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MEMOIRE DE FIN D’ETUDES POUR L’OBTENTION DU DIPLOME DE DOCTEUR EN PHARMACIE

THÈME:

Study of Receptors Involved in Anticancer Therapy.

Présenté par :
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Soutenu le: **10th July 2025**

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Allow us to honor the president and the members of the jury.

President: Dr. Benghanem Soumia: Senior Lecturer Medicinal Chemistry.

Members of the jury:

Dr. Mohammed Nassim BOUKLI HACENE: Senior Lecturer Clinical Pharmacy

Dr. Gendouz Abdou: Assistant Lecturer Medicinal Chemistry

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DEDICATIONS

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Our Lord, grant us good in this world and good in the Hereafter, and save us from the torment of the Fire.

Ameen.”

Mustafa Fadhil Swaleh Said.

Table of Contents

Table of Contents

List of Figures

List of Tables

List of Abbreviations

1. A2AR: Adenosine A2A Receptor
2. ACT: Adoptive T Cell Therapy
3. ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity
4. ADT: Androgen Deprivation Therapy
5. AFP: Alpha-Fetoprotein
6. AKT: Protein Kinase B
7. ALK: Anaplastic Lymphoma Kinase
8. Apaf 1: Apoptotic Protease Activating Factor 1
9. AR: Androgen Receptor
10. BAK: Bcl-2 Antagonist/Killer
11. BAX: Bcl-2-associated X protein
12. BCL-2: B-cell Lymphoma 2
13. BCL-XL: B-cell Lymphoma Extra Large
14. BCR-ABL: Breakpoint Cluster Region - Abelson murine leukemia viral oncogene homolog
15. BH3: Bcl-2 Homology 3
16. BID: BH3 Interacting Domain Death Agonist
17. BMS: Bristol Myers Squibb
18. BRAF: v-Raf murine sarcoma viral oncogene homolog B1
19. BRCA1: Breast Cancer 1
20. BRCA2: Breast Cancer 2
21. CA 19-9: Cancer Antigen 19-9
22. CARD: Caspase Recruitment Domain
23. CBC: Complete Blood Count
24. CD: Cluster of Differentiation
25. CEA: Carcinoembryonic Antigen
26. c-MET: Mesenchymal-Epithelial Transition factor
27. CT: Computed Tomography
28. CTL: Cytotoxic T Lymphocyte
29. CTLA-4: Cytotoxic T-lymphocyte associated protein 4
30. DISC: Death-Inducing Signaling Complex
31. DNA: Deoxyribonucleic Acid

32. DNMT3A: DNA (cytosine-5)-methyltransferase 3 alpha
33. EBRT: External Beam Radiation Therapy
34. EGFR: Epidermal Growth Factor Receptor
35. EMT: Epithelial to Mesenchymal Transition
36. ER: Estrogen Receptor
37. ERK: Extracellular Signal-Regulated Kinase
38. FAS: Fas cell surface death receptor
39. FEP: Free Energy Perturbation
40. FGF: Fibroblast Growth Factor
41. G1/S: Gap 1 / Synthesis phase transition
42. H-bond: Hydrogen Bond
43. HER 2: human epidermal growth factor receptor 2
44. HIF-1 α: Hypoxia-Inducible Factor 1 alpha
45. HNPCC: Hereditary Non-Polyposis Colorectal Cancer
46. IBD: Inflammatory Bowel Disease
47. IC_{50}: Half Maximal Inhibitory Concentration
48. ICAM-1: Intercellular Adhesion Molecule 1
49. ICIs: Immune checkpoint inhibitors
50. ICOS: Inducible T-cell CO-Stimulator
51. IFN-α: Interferon alpha
52. IFN-γ: Interferon gamma
53. Ig C: Immunoglobulin Constant
54. Ig V: Immunoglobulin Variable
55. IGF-1R: Insulin-like Growth Factor 1 Receptor
56. IL: Interleukin
57. ITIMs: Immunoreceptor Tyrosine-based Inhibitory Motifs
58. JAK: Janus Kinase
59. kcal/mol: Kilocalories per mole
60. K_d: Dissociation Constant
61. KRAS: Kirsten Rat Sarcoma viral oncogene homolog
62. mAb: Monoclonal Antibody
63. MAPK: Mitogen-Activated Protein Kinase
64. MD: molecular dynamics
65. MDM2: Mouse Double Minute 2 homolog

66. MDSCs: Myeloid-Derived Suppressor Cells
67. MEK: MAPK Kinase.
68. MHC: Major Histocompatibility Complex
69. MLH1: MutL Homolog 1
70. MM/GBSA: Molecular mechanics with generalized Born and surface area solvation.
71. MMPBSA: Molecular mechanics Poisson-Boltzmann surface area
72. MOMP: Mitochondrial Outer Membrane Permeabilization
73. MRI: Magnetic Resonance Imaging
74. MSH2: MutS Homolog 2
75. mTOR: mechanistic Target of Rapamycin
76. NK: Natural Killer
77. NSCLC: Non-Small Cell Lung Cancer
78. P53: protein 53
79. PD-1: Programmed cell death protein 1
80. PDB: Protein data bank
81. PDGF: Platelet-derived Growth Factor
82. PD-L1: programmed death ligand 1
83. PET: Positron Emission Tomography
84. PI3K: Phosphatidylinositol 3-Kinase
85. PIK3CA: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
86. PSA: Prostate-Specific Antigen
87. PTEN: Phosphatase and Tensin Homolog
88. PubMed: Public/Publisher MEDLINE
89. RAF: Rapidly Accelerated Fibrosarcoma
90. RAS: Rat sarcoma viral oncogene homolog
91. RB1: Retinoblastoma 1
92. RevMan: Review Manager
93. RMSD: Root mean square deviation
94. RTK: Receptor Tyrosine Kinase
95. SERDs: Selective Estrogen Receptor Degraders
96. SERMs: Selective Estrogen Receptor Modulators
97. SHP-2: Src Homology region 2-containing Protein Tyrosine Phosphatase 2
98. SMIs: small molecule inhibitors
99. SMKI: Small Molecule Kinase Inhibitor

100. STAT3: Signal Transducer and Activator of Transcription 3
101. TAMS: Tumor-Associated Macrophages
102. tBID: Truncated BID
103. TCR: T Cell Receptor
104. TGF-β: Transforming Growth Factor beta
105. TKI: Tyrosine Kinase Inhibitor
106. TME: Tumor micro environment
107. TNBC: Triple-Negative Breast Cancer
108. TNF-α: Tumor Necrosis Factor alpha
109. TP53: Tumor Protein 53
110. TRAIL: TNF-Related Apoptosis-Inducing Ligand
111. Tregs: Regulatory T cells
112. TSG: Tumor suppressor genes
113. V600E: Valine (V) to Glutamic Acid (E) substitution at position 600
114. VCAM-1: Vascular Cell Adhesion Molecule 1
115. VEGFR: vascular endothelial growth factor receptor
116. Wnt/β-catenin: Wingless-related integration site/beta-catenin

INTRODUCTION

Cancer remains one of the leading causes of death worldwide. It is a group of diseases caused by gene mutations that disrupt normal cell regulation, leading to uncontrolled growth, evasions of the immune system and potential metastasis (1). Treatment techniques for cancer have improved over the years with the development of targeted therapies. They aim at molecular receptors and signaling pathways that are overexpressed, mutated or even usurped by cancer cells. Key receptors targeted in anticancer therapy include; Tyrosine Kinase Receptors, such as VEGFR, EGFR and HER2, Hormone Receptors, such as the estrogen and androgen receptors, and Immune Checkpoint Receptors, such as CTLA-4 and PD-1/PD-L1. (2)

Since immune evasion is one of the hallmarks of tumor cells (3), we focused on the PD-1/PD-L1 immune checkpoint axis. This interaction between Programmed Death Ligand-1 (PD-L1), which is overexpressed on tumor cells, and Programmed Death-1 (PD-1) on T-cells is a pivotal immune checkpoint that enables tumors to escape immune killing. Inhibition of this interaction has been a cornerstone of therapy, with immune checkpoint inhibitors (ICIs) demonstrating lasting responses across many forms of cancer. (4)

While Monoclonal Antibodies inhibiting PD-1/PD-L1 have been effective, they are marred by high production cost, lack of oral bioavailability, poor tissue penetration, extended half-life and the potential for immune-related adverse events. This has prompted the quest for small molecule inhibitors (SMIs) of the PD-1/PD-L1 interaction, which could possess better pharmacokinetics, oral administration and enhanced tumor penetration. (5)

Computational approaches such as molecular docking, molecular dynamics (MD) simulations, MM/GBSA binding free energy calculations, and pharmacophore modeling have become invaluable tools for early-stage drug discovery. These techniques offer valuable structural insights into ligand binding, stability and critical interactions. They guide the design of therapeutic candidates, though differences in methodology and evaluation criteria among studies can make objective comparison challenging, pointing to the need for standard protocols and judicious interpretation of computational findings. (6,7)

Although a growing body of computational research has been proposing PD-L1 inhibitors with favorable binding profiles, differences in methodologies and reporting parameters, such as docking scores, MM/GBSA binding free energies, RMSD values, and interaction types, have impeded the comparison and ranking of therapeutic candidates in an unbiased way (8).

This thesis addresses this shortfall by conducting a comprehensive meta-analysis of in silico data on PD-L1 inhibitors in an attempt to discern overarching structural and energetic trends among top-performing molecules. By compiling and normalizing these data, the study seeks to facilitate the rational design of next generation PD-L1 inhibitors with improved efficacy and specificity.

The present thesis is organized into two main parts;

- Part I: Theoretical part
- Chapter I: Explores cancer in general with definitions, pathophysiology, symptomatology, risk factors, diagnosis and treatment goals.
- Chapter II: Focuses on receptors involved in anticancer therapy with definitions, mechanisms for targeting receptors, examples of some receptors in anticancer therapy and finally a brief explanation of definition and structure of the PD-1/PD-L1 axis.
- Part II: Practical part
- Chapter I: outlines the materials and methods employed in the study
- Chapter II: presents and discusses the results obtained from the research. The results included: number of articles per year, number of ligands per articles, PDB ID analysis, binding free energies analysis, RMSD analysis, recurring amino acids and finally molecular interaction analysis.

The thesis opens with an introduction that sets the context and closes with a conclusion summarizing the key findings and perspectives.

PART I: THEORETICAL PART-LITERATURE REVIEW

CHAPTER I: CANCER

I.1 Cancer

I.1.1 Definition

Cancer is a group of diseases caused by genetic mutations that disrupt normal cell regulation, leading to uncontrolled growth, evasion of immune responses, and potential metastasis. These mutations affect key processes such as the cell cycle, DNA repair, and apoptosis, often involving signaling pathways like MAPK (mitogen-activated protein kinase), PI3K (phosphoinositide 3-kinase) and Wnt/β-catenin (wingless-related integration site/beta-catenin). Around 140 "driver" genes have been identified, though most tumors require only 2–6 such mutations to develop. Commonly affected genes include tumor protein 53 (TP53), breast cancer genes 1 and 2 (BRCA1/2), in hereditary breast and ovarian cancers, and DNA methyltransferase 3A (DNMT3A), which are linked to poor prognosis and typically appear early in tumorigenesis. Unlike normal cells, which undergo a tightly controlled process of growth and differentiation, cancer cells exhibit the ability to proliferate uncontrollably, evade immune detection, and invade surrounding tissues, sometimes metastasizing to distant organs. (1,9)

Cancer can develop in nearly any tissue and is classified by its cell of origin, such as carcinomas (epithelial cells), sarcomas (connective tissue), leukemia (blood cells), and lymphomas (lymphatic tissue). Its heterogeneity, driven by diverse cancer cell populations and the ability to develop treatment resistance, makes it difficult to diagnose and treat effectively (3,10,11). Cancer cells acquire hallmark capabilities, including sustained proliferative signaling, evasion of growth suppressors, resistance to apoptosis, replicative immortality, angiogenesis and the ability to metastasize, that enable them to grow and spread despite the body's defenses (3). To fuel their rapid growth, these cells often undergo metabolic reprogramming, favoring aerobic glycolysis (the Warburg effect) to meet increased energy and biosynthesis demands (12).

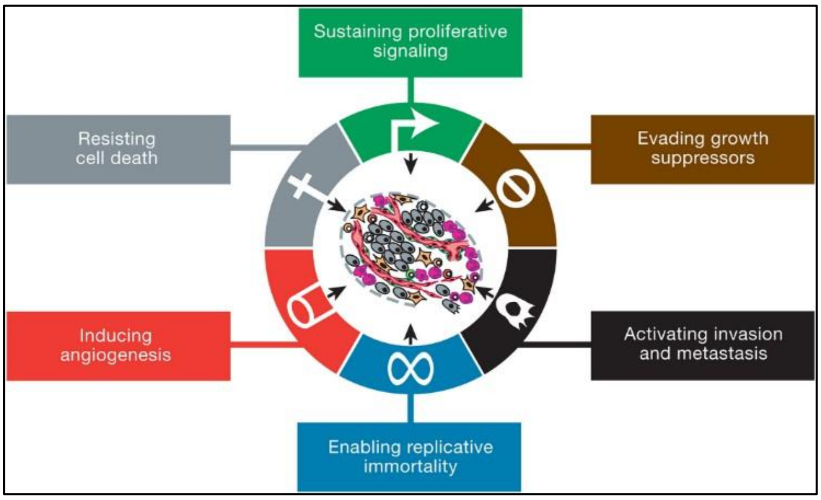


Figure 1: Hallmark capabilities of cancer cells. (3)

A crucial factor in cancer progression is the tumor microenvironment (TME), composed of stromal cells, immune cells, blood vessels, and the extracellular matrix. Tumors manipulate the TME to promote angiogenesis, suppress immune responses, and remodel tissue to facilitate invasion and survival under stress. This dynamic interplay not only drives metastasis and drug resistance but also offers targets for therapy. Recent advances in cancer treatment focus on targeted therapies that inhibit critical molecular pathways and immunotherapies, such as immune checkpoint inhibitors and Chimeric Antigen Receptor (CAR) T-cell therapy, that harness the immune system to fight cancer. These strategies aim to improve treatment precision, overcome resistance, and enhance both survival and quality of life for patients. (10,13)

I.1.2 Cancer pathophysiology and carcinogenesis

The pathophysiology of cancer is complex, involving both genetic and epigenetic alterations that lead to the transformation of normal cells into malignant ones. Central to the formation of cancer is the concept of "hallmarks of cancer" mentioned above. Cancer progression is driven by mutations in oncogenes (e.g., RAS, MYC) that promote uncontrolled cell growth and loss of tumor suppressor genes (e.g., TP53, RB1) that normally regulate cell division and apoptosis. These changes cause genetic instability that drives cancer progression by increasing the rate of mutation and selection of more aggressive clones. (3)

Carcinogenesis is a multistep process involving initiation, promotion, and progression. It is often initiated by a combination of genetic mutations and epigenetic changes, such as DNA methylation and histone modifications, that disrupt normal cell cycle regulation and DNA repair mechanisms. In the **initiation stage**, exposure to carcinogens, such as chemicals, UV radiation, or oncogenic viruses, or inherited mutations causes irreversible genetic damage, transforming normal cells into precancerous ones. During **promotion**, these altered cells undergo clonal expansion, often driven by oncogene activation or loss of tumor suppressor function, leading to further mutation accumulation. In the **progression phase**, cancer cells acquire invasive traits, resistance to apoptosis, and the ability to metastasize. Together, these sequential genetic and epigenetic changes drive the development of a malignant phenotype. (14)

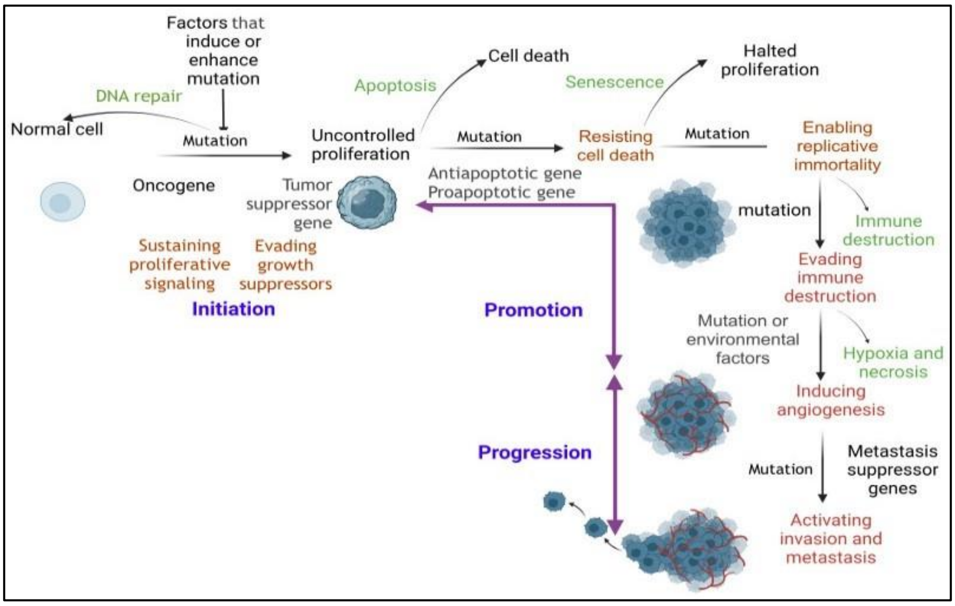


Figure 2: Carcinogenesis. (14)

Mutations in oncogenes and tumor suppressor genes (TSGs) are central to the molecular basis of cancer. Oncogenes originate from proto-oncogenes, normal regulators of cell growth, that, when mutated or overexpressed, promote uncontrolled proliferation and survival. A prime example is the Kirsten Rat Sarcoma viral oncogene homolog (KRAS) G12V mutation, which constitutively activates the RAS/MAPK and PI3K/AKT pathways, driving persistent cell growth in pancreatic, colorectal, and non-small cell lung cancers (NSCLC). (15,16)

Other key oncogenes include EGFR and HER2. EGFR mutations, such as exon 19 deletions and L858R, cause ligand-independent activation in NSCLC and glioblastomas, while HER2 amplification, common in breast cancer, leads to aggressive tumor progression. Similarly, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutations hyper-activate the PI3K/AKT/mTOR pathway and are frequently seen in breast, endometrial, and head and neck cancers. These oncogenic changes have enabled the development of targeted therapies and precision oncology approaches. (15)

Conversely, TSGs function as safeguards by promoting apoptosis, DNA repair, and genomic stability. Mutations or deletions in TSGs remove these controls. TP53, mutated in over 50% of cancers, plays a key role in DNA damage response. TP53, encodes the p53 protein, often referred to as the "guardian of the genome." p53 functions to arrest the cell cycle, repair DNA damage, or trigger apoptosis in response to cellular stress. Its loss impairs apoptosis and facilitates genomic instability. Similarly, Retinoblastoma 1 (RB1) governs the G1/S (Gap 1 / Synthesis phase transition) checkpoint and, when inactivated, as seen in retinoblastoma and small cell lung cancer, leads to unchecked cell cycle progression. Emerging evidence on X-linked TSGs and epigenetic factors such as X- chromosome inactivation has expanded our understanding of TSG regulation beyond the traditional two-hit model. (16,17)

In addition to genetic alterations, chronic inflammation and metabolic reprogramming play crucial roles in cancer progression. The inflammatory tumor microenvironment supports angiogenesis, invasion, and immune evasion, while the Warburg effect, a shift toward aerobic glycolysis, meets the metabolic demands of rapidly proliferating tumor cells. (18).

Together, these alterations in oncogenes and TSGs disrupt cellular homeostasis and drive tumorigenesis. While targeted therapies such as EGFR inhibitors and HER2-directed antibodies have improved outcomes, tumor heterogeneity and drug resistance continue to pose challenges, highlighting the need for continued research into novel mutations and combination strategies. (15,16)

I.1.3 Risk factors and causes

Cancer development arises from a complex interplay of genetic, environmental, and lifestyle factors. Mutations in key genes disrupt normal cellular processes, leading to uncontrolled growth, impaired apoptosis, and genomic instability. (19,20)

Exposure to carcinogens like tobacco smoke and ionizing radiation significantly raises cancer risk. Substances such as benzene and formaldehyde from tobacco smoke can mutate genes like TP53 and KRAS, leading to cancers like lung cancer (21). Ionizing radiation, including ultraviolet (UV) radiation from sunlight, can induce DNA damage such as pyrimidine dimers, increasing the risk of skin cancers, including melanoma (19). Chronic inflammation contributes to cancer development. Conditions like inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, increase colorectal cancer risk by producing reactive oxygen species that damage DNA. (22)

Inherited mutations, such as in BRCA1 and BRCA2 genes, also hinder DNA repair and significantly raise the risk of breast and ovarian cancers (23). Familial adenomatous polyposis (FAP), caused by APC gene mutations, also results in numerous colorectal polyps with high cancer risk (24). Lynch syndrome, or hereditary nonpolyposis colorectal cancer (HNPCC), involves mutations in mismatch repair genes like MLH1 and MSH2, increasing the risk of colorectal and endometrial cancers (25).

Hormonal imbalances also contribute to certain cancers. Prolonged exposure to estrogen, due to factors like early menarche, late menopause, or hormone replacement therapy, increases the risk of breast cancer (26). Elevated testosterone levels have been linked to a higher risk of prostate cancer. (27)

Dietary habits also impact cancer risk. High consumption of red and processed meats is associated with an increased risk of colorectal cancer, possibly due to the presence of carcinogenic compounds formed during meat processing and cooking (28). Conversely, diets rich in fiber, fruits, and vegetables are linked to a reduced risk of various cancers. (29)

In summary, cancer risk is modulated by a combination of genetic susceptibilities and environmental exposures. Understanding these factors is crucial for effective prevention and early detection strategies. (19)

I.1.4 Symptomatology

Cancer presents with a wide spectrum of signs and symptoms arising from both the local effects of tumor growth and systemic responses triggered by malignancy. These manifestations reflect complex pathophysiological processes involving tissue invasion, immune activation, and metabolic disruption, which together shape the clinical presentation and impact quality of life. (3,30,31)

Unexplained weight loss and cancer cachexia, which is characterized by fatigue, muscle atrophy, and appetite loss, are common systemic symptoms. Driven by pro-inflammatory cytokines from immune and tumor cells, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), this syndrome suppresses appetite, promotes catabolism, and interferes with metabolism. Another prevalent condition is anemia, which is frequently caused by tumor-induced impaired erythropoiesis or chronic blood loss. (32,33)

When adjacent tissues are invaded or compressed, local tumor effects occur, resulting in symptoms like pain, nausea, or bowel abnormalities. Neuropathic pain may result from nerve involvement, and thrombosis or bleeding may be brought on by vascular invasion. These effects reflect the tumor’s mechanical and biochemical disruption. (34,35)

Systemic signs like fever also reflect the immune response to tumors, driven by pyrogens such as IL-6 and TNF-α. Anemia, common in cancers like colorectal, often results from occult bleeding or marrow suppression. Lymphadenopathy may indicate immune activation or metastasis, aiding cancer diagnosis. (33,35)

About one in four cancer patients, particularly women and those with ovarian, breast, or lung cancer, experience psychological symptoms like anxiety and depression. These problems lower quality of life and increase the overall burden of symptoms. (36)

These symptoms reflect the systemic reactions of the body as well as the local effects of the tumor. In cancer care, an understanding of this interaction is essential for diagnosis, symptom management, and improving quality of life. (30,31,37)

I.1.5 Cancer Diagnosis

Cancer diagnosis involves imaging, biopsy analysis, and molecular testing to detect mutations or biomarkers. This multidisciplinary approach is essential for accurate classification, staging, and treatment planning (38).

I.1.5.1 Diagnostic Modalities

I.1.5.1.1 Imaging Techniques

Imaging is key for detecting tumors, assessing spread, and guiding treatment. Common methods include: (39)

- **X-ray**: Used for lung cancer and bone metastases.
- **Computed Tomography (CT)**: High-resolution images for staging thoracic, abdominal, and pelvic cancers.
- **Magnetic Resonance Imaging (MRI)**: Excellent soft tissue detail; useful in brain, spine, musculoskeletal, and prostate cancers.
- **Positron Emission Tomography with CT (PET/CT)**: Detects high metabolic activity, aiding in diagnosis and biopsy planning, especially for lymphoma, non-small cell lung cancer, and gastrointestinal cancers.

I.1.5.1.2 Biopsy

A biopsy is the gold standard for confirming cancer, involving tissue removal for microscopic analysis. It reveals tumor type, grade, and cell differentiation. Techniques like immunohistochemistry and molecular testing further guide treatment. Common biopsy types include fine-needle aspiration, core needle, incisional, and excisional biopsies. (40)

I.1.5.1.3 Molecular Testing

Molecular testing is essential in precision oncology, identifying genetic alterations that influence prognosis and therapy selection. For instance, EGFR mutations and ALK rearrangements in NSCLC guide the use of tyrosine kinase inhibitor use; HER2 amplification in breast cancer supports trastuzumab therapy; and BRAF V600E mutations in melanoma help determine targeted therapy options. (1)

I.1.5.1.4 Blood Tests

Blood tests support cancer diagnosis by assessing overall health and tumor activity. These include:(41)

- **Complete blood count (CBC)**: detects anemia or leukocytosis often associated with malignancy or bone marrow infiltration.
- **Liver and renal function tests**: Assess organ function before initiating systemic therapy.
- **Tumor markers:** Substances like alpha-fetoprotein (AFP) for hepatocellular carcinoma, CA 19-9 for pancreatic cancer, PSA for prostate cancer, and CEA for colorectal cancer, help support diagnosis, monitor therapy, and detect recurrence.

I.1.6 Treatment

The main treatment strategies for cancer include surgery, radiotherapy, and systemic therapies, each playing a specific role either alone or in combination. (42)

I.1.6.1 Surgery

Surgery is generally the first therapy for early solid tumors, aiming to remove the tumor with clear margins. Surgery can cure localized cancer and is also used to stage and diagnose. Tumor reduction surgery can be done to reduce tumor size before other therapy. (42)

I.1.6.2 Radiotherapy

Radiotherapy uses high-energy radiation, such as X-rays, gamma rays or protons, to damage cancer cell DNA, blocking division and causing cell death. It targets tumors locally while sparing nearby healthy tissue. (43)

There are two main types:(43,44)

- **External Beam Radiation Therapy (EBRT):** most common, delivers radiation from outside the body directly to the tumor, often used in breast, prostate, lung, and brain cancers.
- **Internal Radiation Therapy (Brachytherapy):** involves placing radioactive sources inside or near the tumor. It delivers a high dose of radiation to a precise area, commonly used in cervical, prostate, and breast cancers.

Radiotherapy can be curative, adjuvant (post-surgery), neoadjuvant (pre-surgery), or palliative (to relieve symptoms in advanced cases). (43)

I.1.6.3 Systemic Therapies

Systemic therapies, which circulate throughout the body, are essential for treating cancers that have metastasized or carry a high risk of spreading. Chemotherapy utilizes cytotoxic drugs to kill rapidly dividing cells but lacks specificity, often damaging healthy cells and causing significant side effects such as fatigue, immunosuppression, and nausea. In contrast, targeted therapies, such as tyrosine kinase inhibitors and monoclonal antibodies, act on specific genetic mutations or molecular pathways that drive tumor growth. These therapies offer key advantages over chemotherapy, including greater selectivity, reduced toxicity, improved tolerability, and efficacy in biomarker-defined subgroups. They also help overcome drug resistance when combined with other agents or next-generation inhibitors. Immunotherapy, particularly immune checkpoint inhibitors like anti-PD-1, anti-PD-L1, and anti-CTLA-4, continues to revolutionize treatment by reactivating the immune system to kill cancer cells. This approach has yielded long-lasting responses in several cancers, including melanoma and non-small cell lung cancer, offering a very promising choice for patients who may not respond to conventional treatments. (45–47)

I.1.7 Treatment Goals

Cancer treatment is guided by tumor type, location, genetic and molecular features, as well as patient specific factors like age and health. The main goals are to eradicate the tumor, prevent metastasis and protect surrounding healthy tissue (3,48–53). The broad objectives generally include the following:

I.1.7.1 Eradication of the Tumor

The main goal of cancer treatment, especially in early stages, is to completely eliminate cancer cells. This may be accomplished through surgery or localized radiotherapy, which targets tumors while sparing healthy tissue. When needed, systemic therapies like chemotherapy, immunotherapy, or targeted therapy are added. Achieving complete remission is the first step toward a potential cure. (48)

I.1.7.2 Prevention of Cancer Spread (Metastasis)

Metastasis is the leading cause of cancer death. To prevent it, systemic therapies like chemotherapy, immunotherapy and targeted drugs are used. Examples include: adjuvant chemotherapy and checkpoint inhibitors. (3)

I.1.7.3 Prevention of Recurrence

Even after tumor removal, microscopic residual disease can cause recurrence. Adjuvant therapies, like chemotherapy, radiotherapy, hormonal therapy, or targeted drugs, are used to eliminate residual cancer cells. For example, hormone therapies treat receptor-positive breast cancer, while trastuzumab targets HER2-positive cases. Lung cancer patients with EFGR mutations may respond to drugs like gefitinib, though resistance can occur. Immunotherapy is also being explored to maintain remission and prevent relapse. (50,51)

I.1.7.4 Improving Survival Rates

Increasing overall and progression-free survival is key, especially in advanced cancers. While a cure may not be possible, modern treatments can extend life with preserved quality. Advances like tyrosine kinase inhibitors(TKIs), monoclonal antibodies, and immune checkpoint inhibitors have improved outcomes in cancers such as lung cancer, melanoma, and blood cancers. (52)

I.1.7.5 Symptom and side effect management

Symptom and side effect management are vital, especially in advanced cancer. Palliative care improves comfort, dignity and survival by relieving patients from pain, fatigue, nausea, and emotional distress, and can be integrated early along with therapy. Modern oncology uses personalized approaches, including tumor genomics and pharmacogenomics, to prevent side effects like neuropathy and fatigue. Supportive therapy, such as, pain management, antiemetics, and growth factors, helps tolerance of the patient to treatment, with quality of life as a significant treatment goal in addition to survival. (49,53)

CHAPTER II: RECEPTORS INVOLVED IN ANTI-CANCER THERAPY

II.1 Definition

II.1.1 Ligands:

These are the signaling molecules that bind to receptors, exogenous or endogenous. They can include hormones, neurotransmitters, cytokines and even drugs. (54)

II.1.1.1 Types of Ligands in Anticancer Therapy

a) **Monoclonal Antibodies (mAbs):** Target surface antigens or immune checkpoints. Ex : Nivolumab/Pembrolizumab (PD-1), Atezolizumab (PD-L1). (55)

b) **Small Molecule Inhibitors:** Inhibit intracellular signaling pathways. Ex: Imatinib (BCR-ABL), Erlotinib (EGFR), Sorafenib (VEGFR/RAF). (56)

c) **Cytokines and Immune Modulators:** Stimulate or modulate immune responses. Ex: IL-2, IFN-α. (57)

d) **Hormone Modulators:** Block or mimic hormones in hormone-sensitive cancers. Ex: Tamoxifen (ER), Letrozole (aromatase). (58)

e) **Cancer Vaccines:** Induce immune response against tumor antigens. Ex : Sipuleucel-T (prostate cancer). (59)

f) **Ligand–Drug Conjugates / Targeted Ligands:** Deliver cytotoxic drugs directly to cancer cells. Brentuximab vedotin (CD30), Trastuzumab emtansine (HER2). (60)

II.1.2 Receptors:

Are specialized proteins located on the surface of cells or within their interior that bind to specific molecules (ligands). This binding triggers a series of biological responses, allowing cells to communicate and respond to their environment. (54)

II.1.2.1 Types of Receptors:

There are 2 types of receptors:

- **Cell Surface Receptors**: These are found on the outer membrane of cells and bind to water-soluble ligands (like hormones or neurotransmitters). (54)
- **Intracellular Receptors**: Located inside the cell, these receptors usually bind to lipid-soluble ligands (like steroid hormones) that can pass through the cell membrane. (54)

II.1.2.2 Functions:

- Receptors play a crucial role in various physiological processes, including cell communication, metabolism, growth, and immune responses. (54)
- They facilitate signal transduction, which involves converting extracellular signals into intracellular responses, thereby influencing a cell's behavior. (54)
- Receptors can induce cell growth, division and death; control membrane channels or regulate cell binding. (54)

In cancer, receptors regulate key processes like growth, immune evasion, angiogenesis, and apoptosis. Receptor dysfunction can cause diseases like cancer, diabetes, and autoimmune disorders. (2)

Cancer can result from receptor abnormalities like:(54)

- **Overexpression** (e.g., HER2 in breast cancer) boosting growth signals
- **Mutations** causing constant activation (e.g., EGFR)
- **Autocrine/paracrine signaling** for self-stimulation
- **Altered trafficking** affecting receptor function and promoting progression

By blocking these receptors or their downstream pathways, therapies are able to inhibit tumor progression, trigger immune recognition, or induce apoptosis (2,47,61,62). The section below outlines the major receptor-targeting mechanisms used in anticancer treatment.

II.2 Mechanisms of Action in Targeting Receptors in Anticancer Therapy

II.2.1 Inhibition of Signal Transduction

Cancer cells frequently depend on the continuous activation of specific signaling pathways which are normally inhibited by;

- **Monoclonal Antibodies (mAbs)**

In targeted therapy, monoclonal antibodies block receptor tyrosine kinases (RTKs) like HER2 and EGFR by binding to their extracellular domains, preventing ligand binding, dimerization, and downstream signaling crucial for cancer cell growth. (61)

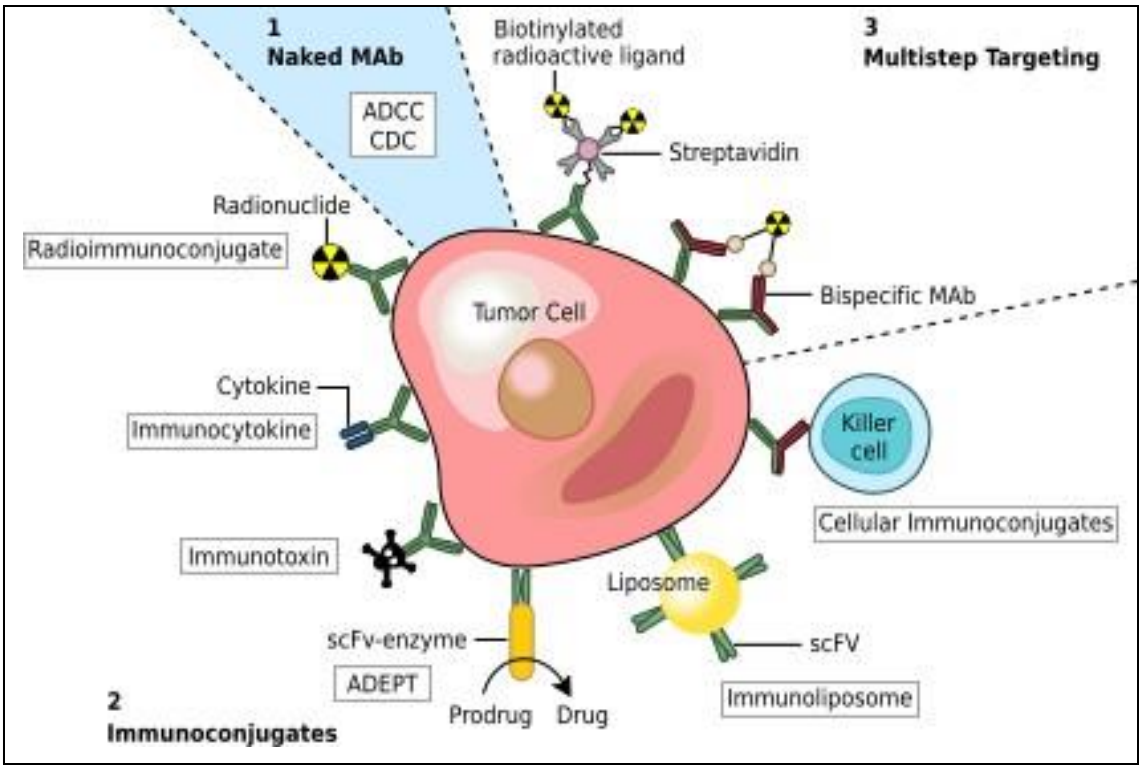


Figure 3: Monoclonal antibodies for cancer (63)

- **Small Molecule Inhibitors (SMIs)**

Small molecule inhibitors block intracellular kinases (enzymes that catalyze the phosphorylation of proteins), disrupting cancer cell growth and survival while minimizing harm to healthy cells. (64)

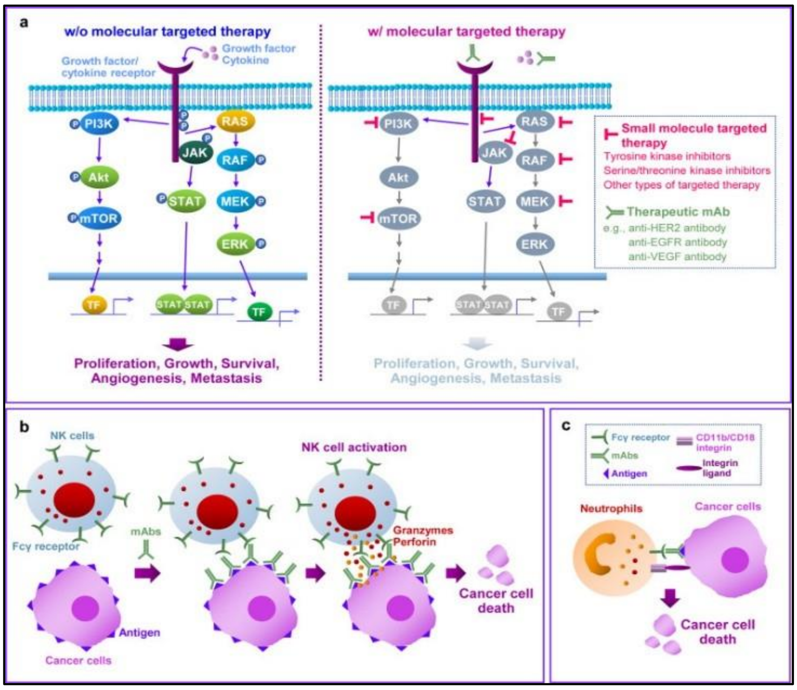


Figure 4: Small molecule targeted therapy. (2)

II.2.2 Antibody-Dependent Cellular Cytotoxicity (ADCC)

ADCC, mostly associated with monoclonal antibody treatments, uses the immune system to eradicate cancer cells. Examples: *Rituximab*: Targets **CD20** on B-cell malignancies and *Trastuzumab*: Targets **HER2** in breast cancer, leading to ADCC and direct apoptosis. (47)

II.2.2.1 Mechanism of ADCC

Monoclonal antibodies (mAbs) are used to target antigens overexpressed on the surface of cancer cells. Once bound, these antibodies help recruit immune effector cells such as natural killer (NK) cells, macrophages, and neutrophils. The Fc region of the antibody interacts with Fc receptors on these immune cells, activating them to release cytotoxic molecules like perforin and granzymes, which induce apoptosis (programmed cell death) in the cancer cells. (47)

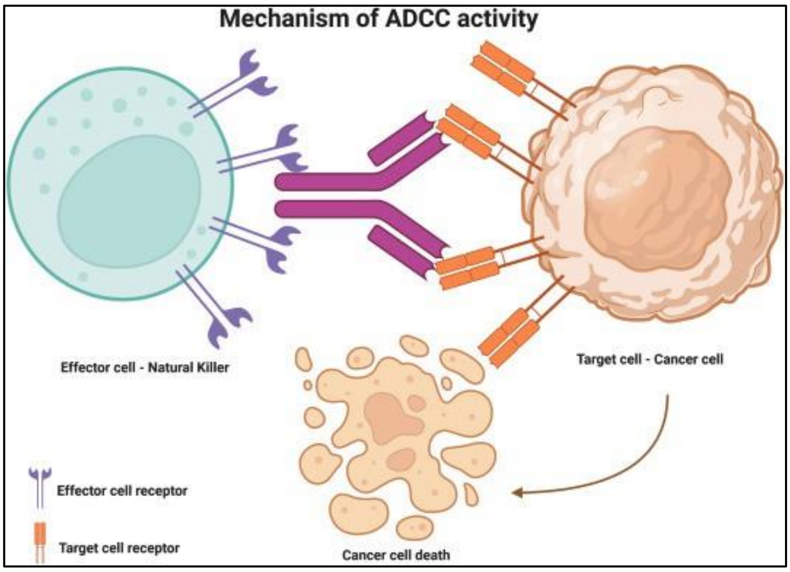


Figure 5: Antibody Dependent Cellular Cytotoxicity in cancer cells. (65)

II.2.3 Angiogenesis Inhibition

Angiogenesis, the growth of new blood vessels, enables tumor growth and metastasis by supplying cancer cells with nutrients and oxygen. (61)

II.2.3.1 Mechanisms of Angiogenesis Inhibition

- **Targeting Vascular Endothelial Growth Factor Receptors (VEGFRs):**

VEGF (Vascular endothelial growth factor) drives angiogenesis via VEGFRs (Targeting Vascular Endothelial Growth Factor Receptors). Inhibitors like bevacizumab block VEGF, while SMKIs like sunitinib and pazopanib inhibit VEGFR signaling, reducing tumor blood supply. (61)

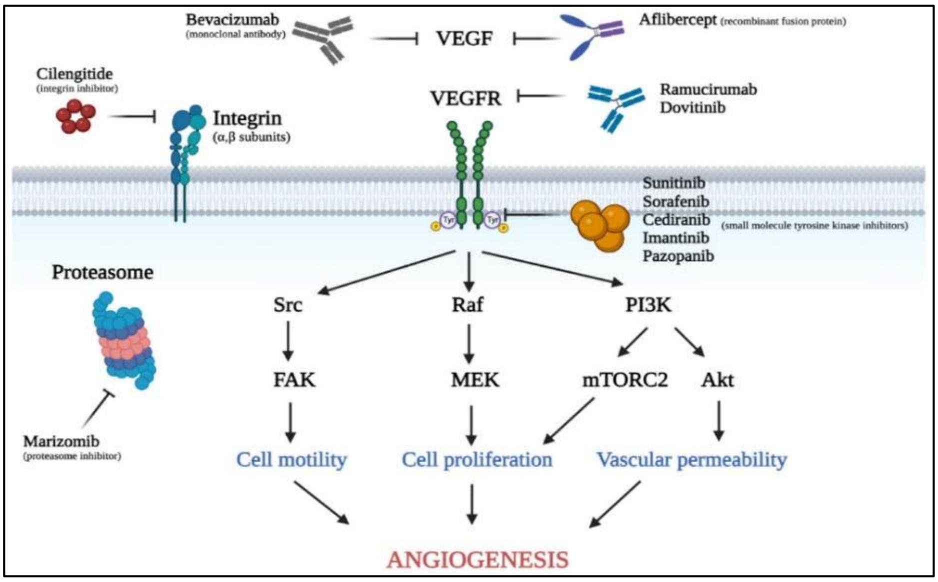


Figure 6: Angiogenesis inhibition (61).

- **Blocking Other Angiogenic Factors:**

Besides VEGF, factors like PDGF (platelet-derived growth factor) and FGF (fibroblast growth factor) drive angiogenesis. Inhibitors like ramucirumab, dovitinib, cilengitide, aflibercept, and marizomib target these pathways, enhancing anti-angiogenic effects. They also normalize the tumor microenvironment, improving immune and drug therapy efficacy. (61,66)

II.2.4 Hormonal Pathway Modulation

Hormonal pathways, especially estrogen and androgens, drive cancers like breast and prostate. (64)

II.2.4.1 Mechanisms of Action

About 70% of breast cancers are estrogen-driven. Treatments include **SERMs (Selective Estrogen Receptor Modulator)** (e.g., tamoxifen), **Aromatase inhibitors** (e.g., letrozole, anastrozole), and **SERDs (Selective Estrogen Receptor Degraders)** (e.g., fulvestrant) to block or degrade estrogen. Prostate cancer therapies target androgen signaling via **Androgen Deprivation Therapy (ADT)** or **AR (Androgen Receptor) antagonists** like enzalutamide. (64)

II.2.5 Blocking Immune Evasion

Tumors evade immune detection, allowing unchecked growth. Targeted therapies counter this, boosting immune recognition and cancer cell destruction. (2). Examples include;

II.2.5.1 Immune Checkpoint Inhibition

This approach blocks the immune checkpoints, boosting the immune system's attack on cancer cells. (2)

Immune checkpoints include :(2)

- **PD-1/PD-L1**: Tumors overexpress PD-L1 to inactivate T cells via PD-1. Inhibitors like pembrolizumab, nivolumab, and atezolizumab block this, reactivating T cells.
- **CTLA-4 (Cytotoxic T-Lymphocyte-Associated Protein 4):** Competes with CD28 to suppress T cell activation. Tumors use this to escape immunity. Ipilimumab blocks CTLA-4, boosting T cell response and anticancer activity.

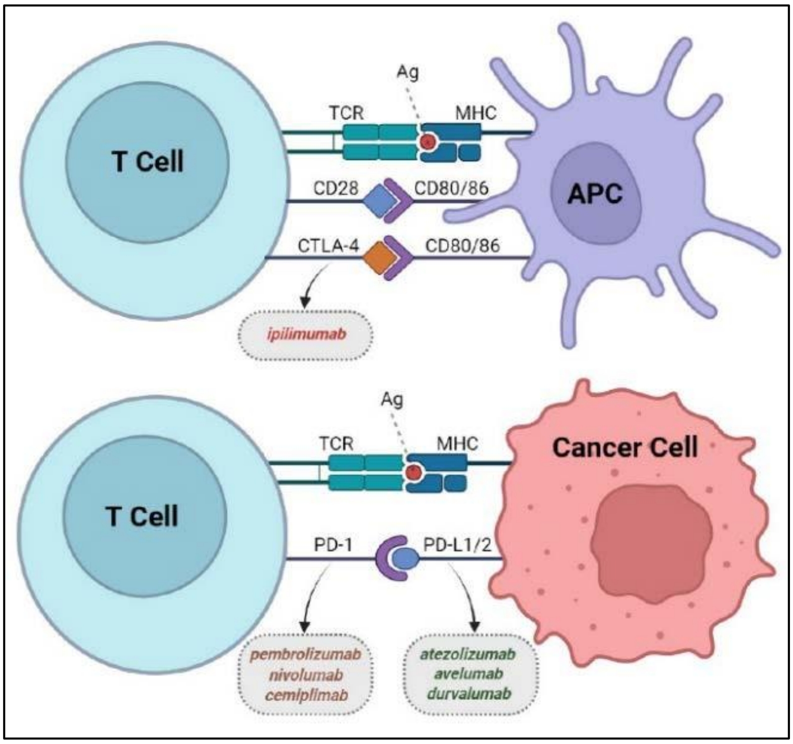


Figure 7: Immune checkpoint inhibition. (2)

II.2.5.2 Restoring antigen presentation

Tumors evade immunity by disrupting antigen presentation, often through MHC downregulation or pathway mutations. Enhancing antigen presentation boosts immune recognition and cancer cell elimination (67). Strategies include:

- **Interferon therapy:** Cytokines like IFN-γ boost tumor visibility by increasing MHC I expression and activating antigen-presenting cells. (67)
- **Epigenetic modifiers:** Epigenetic drugs like decitabine, vorinostat, and JQ1 boost antigen presentation and MHC expression, enhancing immune recognition of tumors.

Targeting epigenetic regulators also improves immune cell function, increasing anti-tumor responses. (68)

- **Adoptive T cell therapy (ACT):** This approach involves isolating a patient's T lymphocytes, then expanding or genetically modifying them to better target tumor antigens. Examples include Chimeric Antigen Receptor (CAR) T cell therapy, which modifies T cells to target antigens like CD19, and Tumor Infiltrating Lymphocyte (TIL) therapy, which expands tumor-infiltrating T cells before reinfusion. (67)

II.2.5.3 Targeting Immunosuppressive Cytokines

Cytokines like transforming growth factor-beta (TGF-β), interleukin-10 (IL-10), and vascular endothelial growth factor (VEGF) promote an immunosuppressive tumor microenvironment by increasing regulatory T cells, myeloid-derived suppressor cells, and inhibiting dendritic cells. Targeting these cytokines with agents like galunisertib (anti TGF-β) and bevacizumab (anti-VEGF) enhances antigen presentation and T cell activation. (69)

II.2.5.4 Depleting Immunosuppressive Cells

These therapies reduce immunosuppressive cells like regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), enhancing cytotoxic T lymphocyte (CTL) and natural killer (NK) cell function. (70). Examples include:

- **Anti-CCR4 antibodies** (e.g., mogamulizumab) to deplete Tregs. (70)
- **CSF-1R inhibitors** (e.g., pexidartinib) to block MDSC and TAM development. (71)
- **Chemotherapeutics** (e.g., low-dose cyclophosphamide, gemcitabine, 5-FU) to selectively eliminate Tregs and MDSCs, boosting effector immune responses. (72)

II.2.5.5 Overcoming Hypoxia in Tumor Microenvironment

Hypoxia in the tumor microenvironment promotes angiogenesis, immune suppression, and metastasis through HIF-1α activation and PTEN inhibition. Although the exact mechanisms remain unclear, normalizing tumor vasculature improves oxygenation, restores PTEN activity, reduces HIF-1α, and revives immune function. (73). Key strategies include:

- **Vascular normalization:** Antiangiogenic therapies restore vessel function, improve oxygen delivery, and enhance immune cell infiltration via ICAM-1/VCAM and PTEN activation.

- **Targeting HIF-1α pathways:** Inhibiting HIF-1α and its downstream effectors (VEGF, PD-L1) reduces immune suppression and boosts T cell activity.
- **Blocking adenosine signaling:** Hypoxia increases adenosine, which dampens immunity. Adenosine A2A receptor (A2AR) antagonists can restore immune function. (73)

II.2.6 Induction of Apoptosis

II.2.6.1 Apoptotic Pathways:

- **Intrinsic Pathway (mitochondria-dependent):**

Is triggered by internal stress signals like DNA damage or oxidative stress. It involves mitochondria and is regulated by Bcl-2 family proteins; pro-apoptotic (e.g., Bax, Bak) and anti-apoptotic (e.g., Bcl-2, Bcl-xL). Activation of pro-apoptotic proteins causes mitochondrial outer membrane permeabilization (MOMP), releasing cytochrome c. Cytochrome c binds Apaf-1 to form the apoptosome, which attracts pro-caspase-9 to its caspase recruitment domain (CARD), enabling auto-activation and subsequent proteolysis. This leads to activation of executioner caspases-3, -6, and -7, resulting in apoptotic cell death. (62)

- **Extrinsic Pathway:**

The extrinsic apoptosis pathway is triggered by ligand binding to death receptors (e.g., FAS, TRAIL receptors) on the cell surface, forming the death-inducing signaling complex (DISC) and activating caspases. In some cells, caspase-8 amplifies the signal by cleaving Bid into t-Bid, which activates mitochondrial membrane permeabilization, linking the extrinsic and intrinsic pathways. (62)

- **Caspase Activation:**

Caspases are cysteine proteases essential for apoptosis. Initiator caspases (e.g. caspase -8, -9) activate effector caspases (e.g. caspase -3, -7), which cleave cellular components, leading to hallmark apoptotic changes like cell shrinkage, chromatin condensation, and DNA fragmentation. (62)

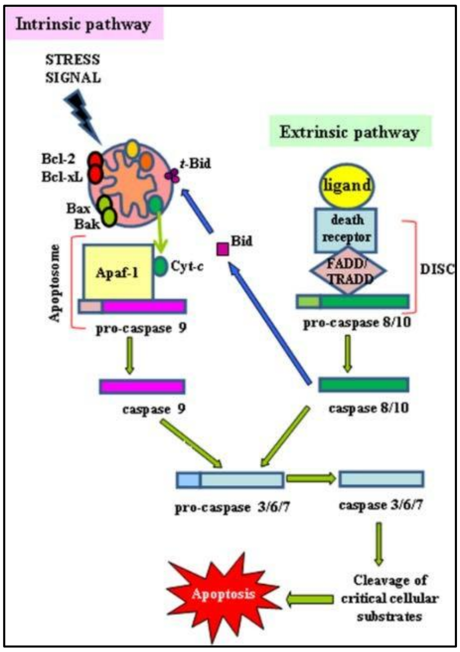


Figure 8: Apoptosis (62)

Therapies include: BH3 Mimetics, Death Receptor Agonists, Restoring p53 Function (62).

II.3 Some receptors involved in anticancer therapy

II.3.1 Growth factor receptors

Growth factor receptors, mainly receptor tyrosine kinases (RTKs), are membrane proteins that bind growth factors to trigger signaling pathways controlling cell growth and differentiation. In cancer, these receptors are often overexpressed, driving uncontrolled proliferation. Targeting them helps block tumor-related signaling, making them key anticancer therapy targets. (74)

II.3.1.1 Tyrosine Kinase Receptors

a) Vascular Endothelial Growth Factor Receptor (VEGFR)

VEGFRs regulate angiogenesis by activating endothelial cell migration, proliferation, and survival upon binding VEGFs, supporting processes like wound healing and vascular repair. In cancer, VEGFR overexpression promotes tumor growth and metastasis through increased blood supply. Therapies include monoclonal antibodies that block VEGFR and tyrosine kinase inhibitors that inhibit its signaling. (75)

b) Epidermal Growth Factor Receptor (EGFR)

EGFR regulates epithelial growth, repair, and immune responses. Upon binding to ligands like EGF or TGF-α, it activates pathways (MAPK, PI3K/Akt, JNK) driving proliferation. In cancer, EGFR is often overexpressed or mutated (e.g., Δ(2–7) EGFR), promoting tumor growth, angiogenesis, and metastasis, making it a key therapeutic target.(76)

c) HER2 (Human Epidermal Growth Factor Receptor 2)

HER2 (ErbB2) is a tyrosine kinase involved in cell growth and survival, especially in breast tissue (77). In cancer, its overexpression promotes proliferation and poor prognosis. Unlike other EGFR family members, HER2 is activated via heterodimerization (mainly with HER3), enhancing oncogenic signaling. (78)

d) Fibroblast Growth Factor Receptor (FGFR)

FGFRs regulate key processes like development, proliferation, wound healing, and angiogenesis. In cancer, FGFR mutations, amplifications, or fusions lead to uncontrolled growth, survival, angiogenesis, and therapy resistance, making FGFRs important therapeutic targets. (79)

e) Insulin-Like Growth Factor-1 Receptor (IGF-1R)

IGF1R is a tyrosine kinase receptor vital for growth, development, metabolism, and tissue repair via PI3K/AKT and ERK pathways. (80). In cancer, it promotes cell proliferation, survival, and anti-apoptosis, often through overexpression and interaction with pathways like p53/MDM2. (81)

II.3.1.2 Non-Tyrosine Kinase Receptors

a) Transforming growth factor beta receptor (TGF-βR)

The TGF-β receptor, a serine/threonine kinase, regulates cell growth, immune response, and tissue repair. In cancer, it acts as a tumor suppressor early on but later promotes progression, EMT (epithelial-to-mesenchymal transition), immune evasion, and metastasis, showing stage-dependent dual roles. (82)

b) NOTCH receptors

Notch receptors regulate cell fate, differentiation, and apoptosis in a context-dependent manner. In cancer, they act as oncogenes or tumor suppressors depending on tissue type, influencing processes like angiogenesis, proliferation, and treatment resistance or promoting differentiation and homeostasis. (83)

c) IL-6 Receptor

The IL-6 receptor (IL-6R) mediates IL-6 signaling, which regulates inflammation, immune response, and tissue repair. In cancer, IL-6R promotes tumor growth, metastasis, and immune evasion by activating pathways like PI3K/AKT and JAK/STAT3, and supporting EMT (epithelial-to-mesenchymal transition) and cancer stem cell renewal. (84)

II.3.1.3 Other Tyrosine Kinase Receptors

a) ALK (Anaplastic lymphoma kinase) receptor. (85)
b) C-MET (Cellular-Mesenchymal Epithelial Transition factor).(86)

II.3.2 HORMONE RECEPTORS

a) Estrogen receptors (ERs)

Estrogen receptors (ERα and ERβ) regulate reproductive health, bone density, cardiovascular function, and immune balance by modulating gene expression. In cancer, especially breast and endometrial, ERs drive tumor growth and therapy resistance through estrogen-mediated cell proliferation and survival. (87)

b) Androgen Receptors (ARs)

ARs regulate male reproductive development, secondary sexual traits, and maintain physiological balance across various systems. In cancer, especially prostate, ARs drive tumor growth via androgen signaling. Mutations or overexpression can lead to castrate-resistant prostate cancer and are also implicated in tumors of the lung, liver, kidney, and bladder. (88)

II.3.3 Serine/Threonine receptors.

BRAF

BRAF is a serine/threonine kinase that activates the MAPK pathway, regulating cell growth and survival. Normally, it transmits signals from RTKs to MEK and ERK to maintain tissue homeostasis. In cancer, mutant BRAF, especially V600E, drives uncontrolled proliferation and therapy resistance by activating the pathway independently of external signals. (89)

II.3.4 IMMUNE CHECKPOINT RECEPTORS

a) CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4)

CTLA-4 is an immune checkpoint receptor on activated T cells that competes with CD28 for B7 ligands on APCs, inhibiting T-cell activation and maintaining immune tolerance. In cancer, CTLA-4 is overexpressed and suppresses anti-tumor T-cell responses, allowing tumor immune evasion**.** (90)

b) PD-1/PD-L1 (Programmed Death Receptor-1/Programmed Death Ligand-1)

The focus of our research is the Programmed Death Ligand-1. The following includes the definitions and structure of the PD-1/PD-L1 axis.

II.4 PD-1/PD-L1 (Programmed Death Receptor-1/Programmed Death Ligand-1)

II.4.1 Definition and mechanism of action.

Programmed Cell Death Protein 1 (PD-1), also known as CD279, is a 50–55 kDa transmembrane glycoprotein expressed on T cells, B cells, and dendritic cells. As a key immune checkpoint, PD-1 regulates T-cell activation and prevents excessive immune responses. It binds to its ligand PD-L1 (CD274), found on antigen-presenting cells and many non-immune cells, including tumor cells. This interaction inhibits the production of cytokines like IL-2, IL-7, IL-10, and IL-12 and recruits SHP-2 (Src Homology region 2-containing Protein Tyrosine Phosphatase-2), a phosphatase that dephosphorylates T-cell receptor (TCR) signaling proteins, thereby reducing T-cell activity. PD-L1 expression is often upregulated by inflammatory signals like IFN-γ, enhancing immune regulation during inflammation.(4,91,92)

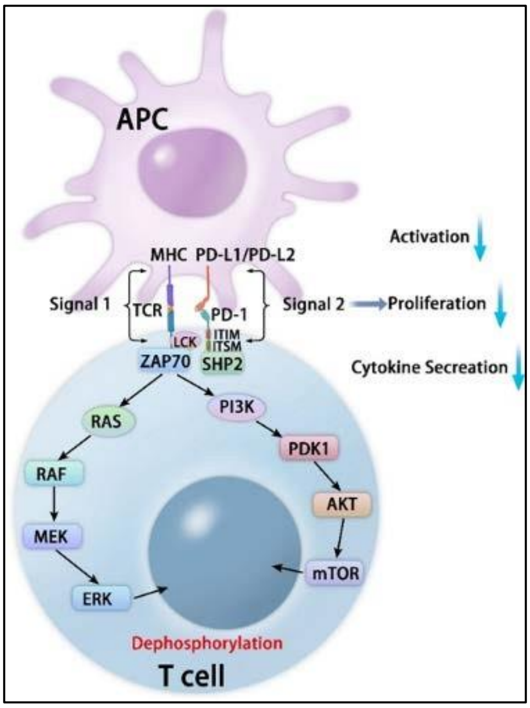


Figure 9: Mechanisms of PD-1-mediated inhibition in T cells. (91)

While essential for maintaining immune tolerance, the PD-1/PD-L1 axis can be exploited by cancer cells to suppress T-cell responses and evade immune detection. High PD-L1 expression in tumors inhibits T-cell cytotoxicity, promoting tumor growth and resistance to treatment. Thus, although this pathway protects against autoimmunity, it also contributes to immune escape in cancer. Targeting PD-1/PD-L1 with immune checkpoint inhibitors has revolutionized cancer therapy by restoring T-cell function and enhancing anti-tumor immunity. (4,91,93)

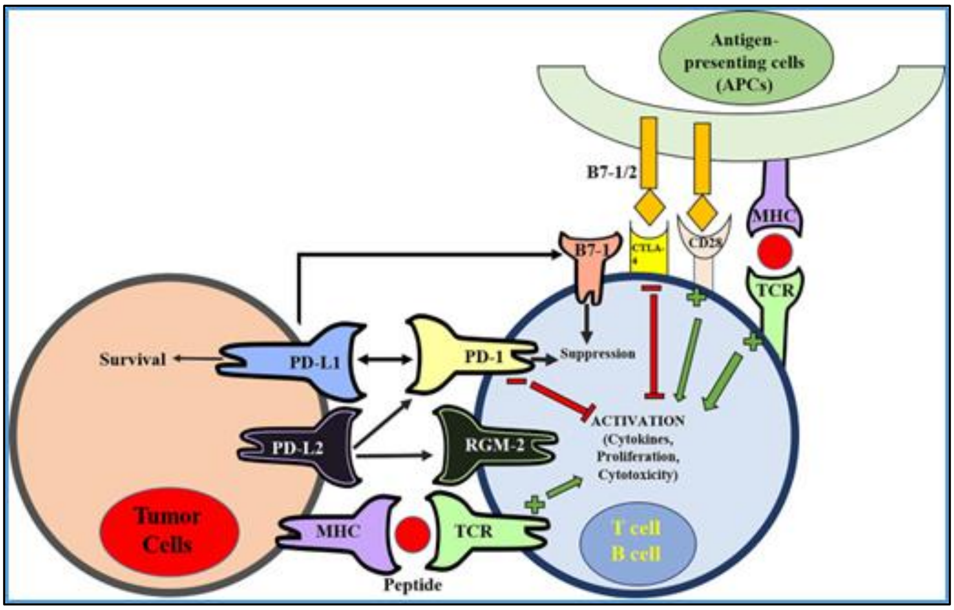


Figure 10 : Demonstration of PD-1/ PD-L1 and CTLA-4 suppressing T cell activation. (94)

II.4.2 Structure of PD-1 and PD-L1

II.4.2.1 Structural Features of PD-1

PD-1 is a transmembrane immune checkpoint receptor that has 288 amino acid residues. PD-1 has a critical role in the regulation of immune responses by inhibiting T cell activity. PD-1 has a basic structure that includes three significant domains: an extracellular immunoglobulin variable (Ig V) domain, a single-pass transmembrane domain, and an intracellular cytoplasmic tail with an immunoreceptor tyrosine-based inhibitory motif (ITIM), which accounts for its inhibitory signal function.(95)

The extracellular domain of PD-1 assumes a β-sheet immunoglobulin variable (Ig V) fold, stabilized by a disulfide bridge between Cys54 and Cys123, characteristic of its structure. Unlike other CD28 family members. CD28, CTLA-4, and ICOS, PD-1 lacks a second disulfide bond present in these molecules. Electron density map analysis reveals a generally well-defined structure, except for a disordered region between the C' and D β-strands. In addition, there is no sign of PD-1 dimerization observed in the crystal lattice, in agreement with findings reporting that PD-1 exists as a monomer in solution, distinct from certain other immune receptors.(95,96)

The Ig V domain of PD-1, is primarily accountable for ligand binding with PD-L1. Several critical amino acid residues participate in this interaction, namely Val64, Asn66, Tyr68, Ser73, Asn74, Gln75, Thr76, Lys78, Val90, Leu122, Gly124, Ile126, Leu128, Pro130, Lys131, Ala132, Ile134, and Glu136. All of these residues are located on the C, C', C", F, and G β-strands, as well as the CC' and FG loops, which altogether contribute a stable framework for ligand binding. A hydrophobic patch containing Val64, Ile126, Leu128, Ala132, and Ile134 constitutes the core of the PD-1/PD-L1 interaction interface and functions as a critical anchor that enhances both affinity and structural stability. (95)

Besides hydrophobic contacts, the PD-1/PD-L1 complex is also stabilized by specific hydrogen bonds and ion bridges, such as those between Thr76 (PD-1) and Tyr123 (PD-L1) and between Gln75 (PD-1) and Arg125 (PD-L1). These are components of high-affinity, specific receptor-ligand interaction, which is essential to PD-1's immune inhibitory function. (95)

II.4.2.2 Structural Features of PD-L1

PD-L1 is a 290-amino acid residue type I transmembrane protein. The domain composition has an extracellular part consisting of two immunoglobulin-like domains (a single Ig V-like and an Ig C-like), a single-pass transmembrane portion, and a short cytoplasmic tail. The centrally located extracellular Ig V-like domain is responsible for exerting PD-L1's immunosuppressive function by binding with PD-1(95).

Key residues located within the extracellular region, Ile54, Tyr56, Met115, Ala121, and Tyr123, directly contribute to PD-L1’s binding interface with PD-1. These residues align with hydrophobic counterparts on the PD-1 molecule (Val64, Ile126, Leu128, Ala132, and Ile134) to form a compact hydrophobic core at the interface, which is essential for a stable and specific interaction. (95)

One interesting feature of the PD-1/PD-L1 interaction is the π-π stacking between Tyr68 of PD-1 and Tyr123 of PD-L1, which enhances the rigidity and binding energy of the complex. This contact, in combination with hydrogen bonds and hydrophobic anchoring, accounts for the formation of an extremely stable receptor-ligand complex. With this structural arrangement, PD-L1 effectively inhibits T cell activation, a mechanism usually exploited by tumor cells to escape immune surveillance. (95)

Further structural analysis has identified three major binding “hotspot” regions on PD-L1 that contribute to its interaction with PD-1: (95)

1. A hydrophobic cavity comprising Tyr56, Glu58, Arg113, Met115, and Tyr123, which facilitates accommodation of Ile134 from PD-1.

2. A second zone composed of Met115, Ala121, and Tyr123 that interfaces primarily with Ile126 on PD-1.
3. A polar groove defined by residues Asp122 through Arg125, and Asp26, engaging in polar interactions with Tyr68, Gln75, and Thr76 from PD-1.

These discrete regions are instrumental in ensuring proper alignment and binding strength between the two proteins, and they also represent potential targets for pharmacologic disruption in checkpoint inhibition therapies.(95)

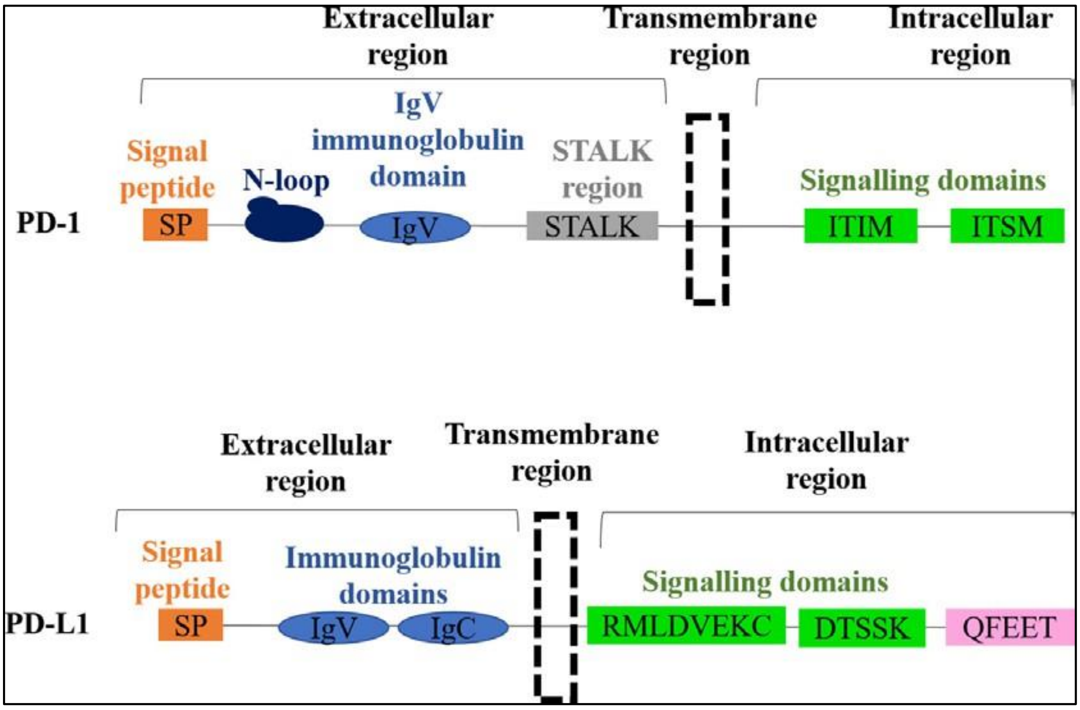


Figure 11: Structure of PD-1/PD-L1.(97)

PD-L1 is usually monomeric under normal conditions but can form homodimers via its IgC domain during membrane clustering or in crystal environments. While not required for PD-1 binding, this dimerization may enhance immune evasion by stabilizing inhibitory signaling and suppressing T cell activity. (98)

Small molecule inhibitors (SMIs), particularly biphenyl-based compounds from the BMS group, have been shown to induce and stabilize PD-L1 homodimers by binding at the dimer interface. This blocks PD-1/PD-L1 interaction indirectly, preventing immune suppression without targeting the PD-1 binding site directly. These SMIs also promote PD-L1 internalization and degradation, enhancing T-cell activation and antitumor immunity. Structural and computational studies have identified key residues and hydrophobic pockets crucial for dimer stabilization, guiding the design of more potent inhibitors. Compared to monoclonal antibodies, SMIs offer advantages such as oral availability, better tumor penetration, and lower production costs, making PD-L1 dimerization a promising strategy in cancer immunotherapy. (99,100)

Anti–PD-1/PD-L1 therapies include: (101)

- Monoclonal Antibodies: anti PD-1 such as Nivolumab, Pembrolizumab, Cemiplimab and anti-PD-L1 such as Atezolizumab, Durvalumab, Avelumab.
- Peptide-Based PD-1/PDL-1 Inhibitors such as AUNP-12.
- Non-peptide small molecule inhibitors: Bristol-Myers Squibb (BMS) compounds such as BMS-8 and BMS-202.
- Aptamer Therapy: where they use Aptamer-Drug Conjugates (APDCs) designed to deliver immunomodulators and block PD-1/PD-L1 interactions.

Cancers that overexpress PD-1 and PD- L1 include: Melanoma, Non-Small Cell Lung Cancer (NSCLC), Renal Cell Carcinoma, Bladder Cancer, Triple-Negative Breast Cancer (TNBC), Hodgkin Lymphoma, Primary Mediastinal B-cell Lymphoma, Squamous Cell Carcinomas of the Vulva and Cervix, Gastric Cancer, Esophageal Cancer. (102)

Although none have FDA approval yet, several small molecule PD-L1 inhibitors are in preclinical and early clinical trials. These include:

- Bristol-Myers Squibb compounds (Preclinical): used in crystallographic studies. (103)
- CA-170 (Clinical phase 2b/3): dual PD-L1/VISTA antagonist. (104)
- INCB086550 (Clinical phase 2): oral PD-L1 inhibitor. (104)
- MAX-10181 (Clinical phase 1): selective oral PD-L1 inhibitor. (104)
- IMMH-010 (Clinical Phase 1): oral PD-L1 inhibitor. (104)

PART II: PRACTICAL PART

CHAPTER I：MATERIAL AND METHODS

Objectives

The objective of this study is:

- To facilitate the rational design of next generation PD-L1 inhibitors with enhanced efficacy and specificity by performing a systematic meta-analysis on computational studies of PD-L1 inhibitors.

To achieve this, we aimed to:

- Compare and evaluate the binding affinities and RMSD values of known PD-L1 inhibitors base on molecular docking results.
- Analyze key molecular interactions (such as hydrogen bonds, π-π stacking and hydrophobic contacts) and identify conserved PD-L1 binding site residues.
- Identify and categorize recurring substituents and structural motifs (such as halogenated phenyl groups, ether bridges, alkyl tails) that contribute to ligand stability and orientation.
- Highlight interaction patterns and structural features that could inform the design of novel, selective PD-L1 inhibitors.

I.1 Introduction

We conducted a meta-analysis to gather and analyze data from multiple studies with the primary objective of reviewing various articles on research platforms such as PubMed and Google scholar to do an assessment of the study of PD-1/PD-L1 receptors in anti-cancer therapy. The secondary objectives included evaluating molecular studies of PD-L1 inhibitors, comparing their binding affinities, availability of RMSD values, key interaction residues and identification of consistent patterns.

The selection of studies was done according to predefined criteria. Meta-analysis is a method of synthesis of quantitative data from multiple studies addressing a similar research question. Meta-analyses are often, but not always, important components of a systematic review. (105)

The seven steps criteria used when conducting a meta-analysis research;

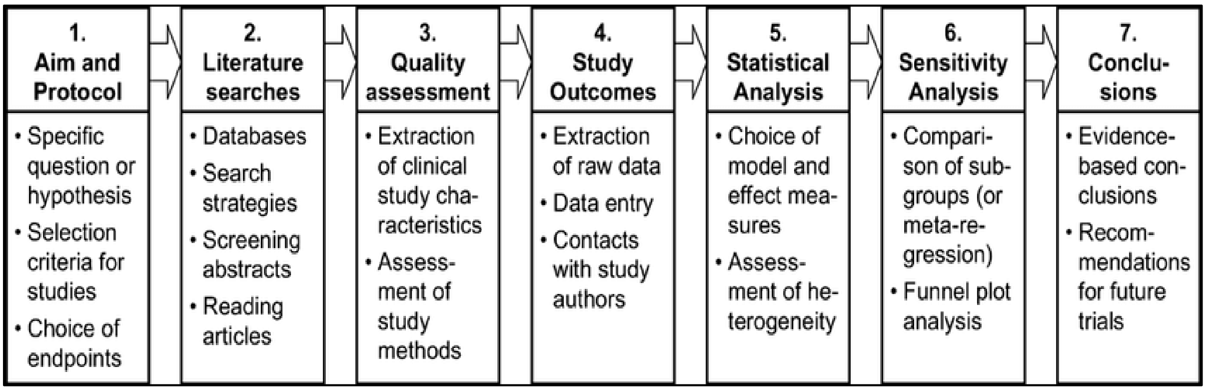


Figure 12: Steps for conducting a meta-analysis. (106)

I.2 Material and Methods

- **Search engines:** Pub med, Google scholar, Research gate, Science direct, Pub chem.
- **Key words:** PD-1/PD-L1, immune checkpoint, docking, molecular interaction, anticancer therapy, in silico drug design
- **Search strategy:** The results found on PubMed were 3 articles after using filters which were articles submitted within a five-year range (2019-2025), articles with full text available in English language. On science direct we found 62 articles after application of filters which are articles from 2019 -2015 available in English language.

On google scholar we found 17,000 articles before using filters as majority did not have full text available. After filters we had 49 articles. Research gate was also used to extract 30 articles. The titles and key words of the articles identified as potentials were selected for examination. We conducted a comprehensive analysis of the full text to assess their validity and availability of crucial information. This took a period of 4 months (February to May 2025)

We ended up narrowing our research to 27 articles. The literature research was independently taken and assessed by the following authors; M.A and M.F.

- **Interpretation**

For data analysis and interpretation of the data collected, **Microsoft Excel 2016 and Microsoft Word 2016** were utilized for data entry, preliminary analysis and tabulation. For meta analytic visualization, specifically to create the forest plot, **Review Manager (RevMan) 5.4** was used. The software permitted the calculation of effect sizes and confidence intervals and the graphical presentation of the study.

I.3 Criteria of evaluation

I.3.1 Inclusion criteria

Articles were selected according to the following;

- Original full-text articles published in English within a six year range 2019-2025. Selection of the most recent articles.
- Molecular docking study of PD-1/PD-L1 inhibitors in anti-cancer therapy
- Availability of RMSD values and binding free energy calculated using MMGBSA/MMPBSA in kcal/mol.
- Structure of the inhibitors studied.
- Identification of key residues in the interaction with PD-1/PD-L1 pathway.
- Description of molecular bonds involved in ligand/receptor interaction

I.3.2 Exclusion criteria

The following articles were excluded;

- Articles with no full text availability, payment requirements and limited preview
- Articles not published within a range of 6 years
- Articles mentioning other receptors apart from the specified PD-1/PD-L1 receptor
- Energy calculated in other forms
- Inhibitors that are immunology related (antibodies)

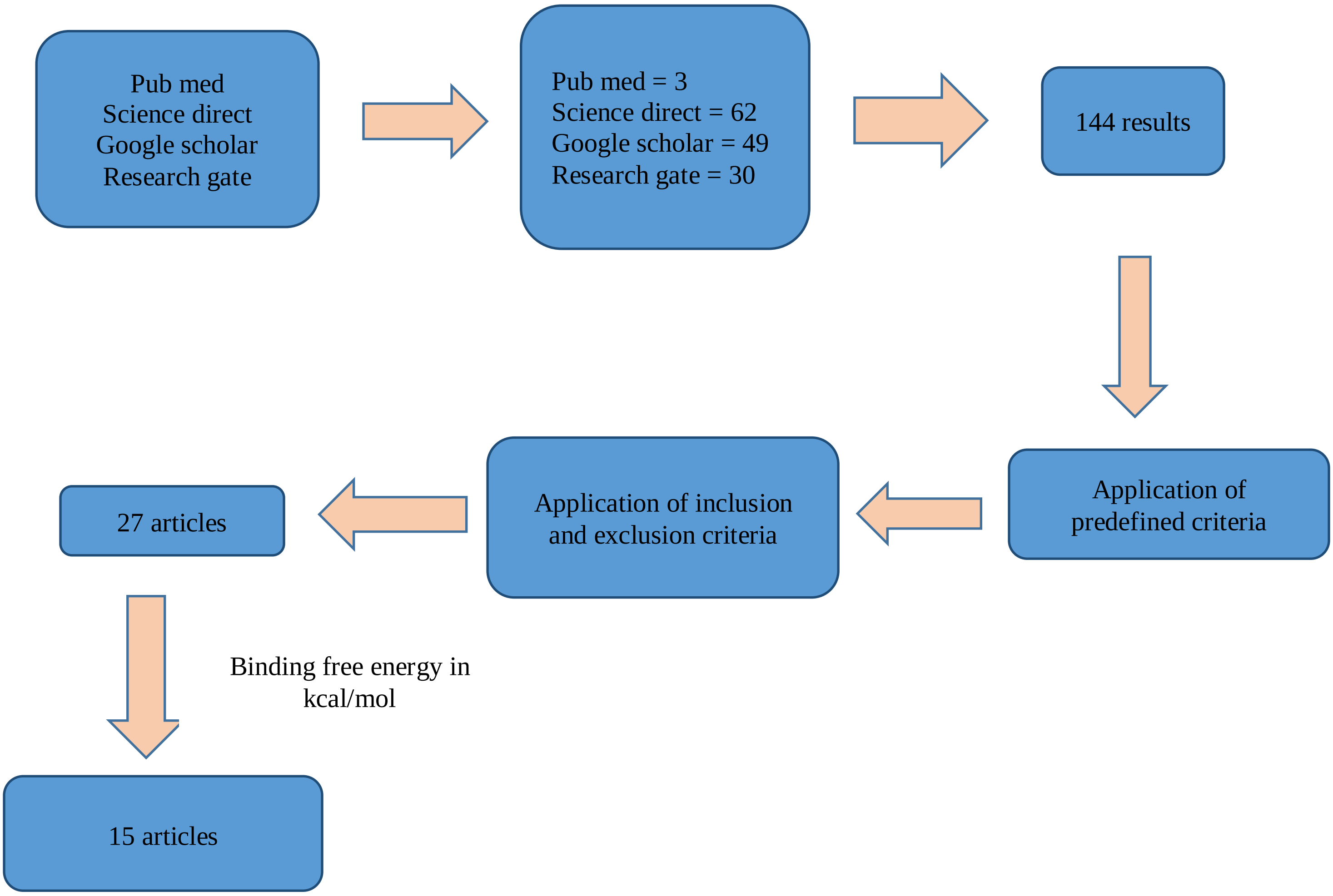


Figure 13: Diagram illustrating the flow chart of the study and the inclusion criteria

CHAPTER II: RESULTS AND DISCUSSION

II.1 Results

Our research on the databases: PubMed, Science Direct, Google Scholar and Research Gate, yielded a total of 144 results. Of these, 27 articles were included, and 117 were excluded following a meticulous review of article titles, thorough examination of abstracts and full text review.

The included articles contained comparative analysis of structures of 69 ligands binding to PD-L1, binding energies, docking performance such as RSMD (Root Mean Square Deviation) of the crystal structures used in Molecular Docking, residues involved in the binding of these ligands to PD-L1 and the different types of interactions involved. Data were compiled to evaluate the efficacy and recurring features of potent ligands.

II.1.1 Number of articles per year.

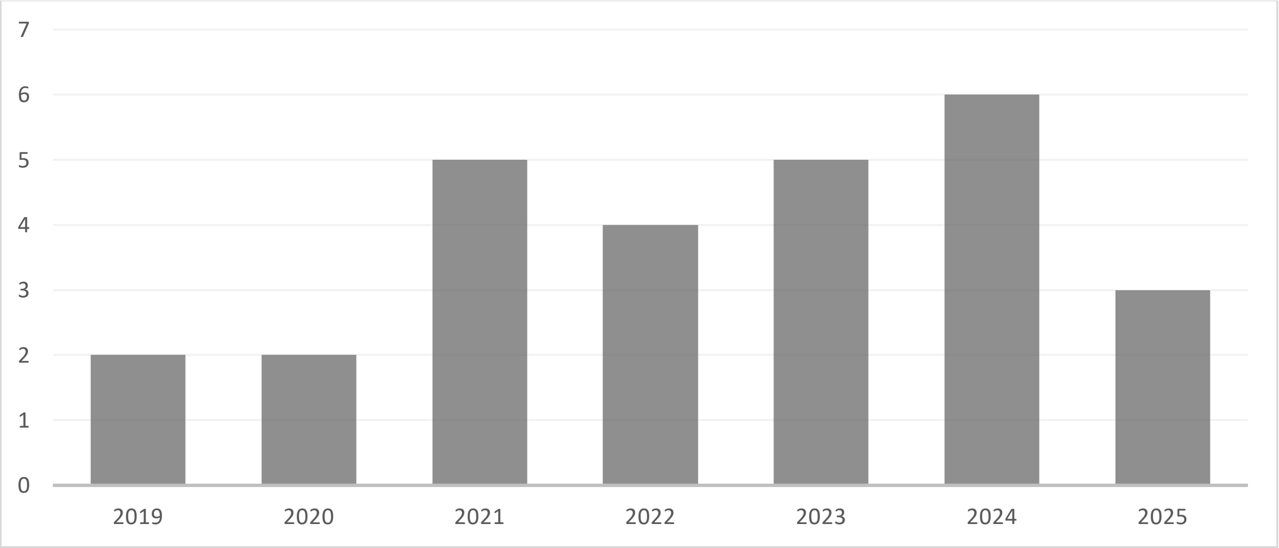


Figure 14: Graph representing number of articles for each year.

An analysis of article distribution for different years showed a growing trend in publications pertaining to the design of PD-L1 inhibitors. With a total of 27 papers, the earliest study was from 2019. There was a noticeable increase starting from 2021, with 5 articles, and a peak in 2024, with 6 articles. Given the clinical effectiveness of checkpoint inhibitors, this trend reflects the increased scientific interest in using cancer immunotherapy to target immune checkpoints like PD-L1.

II.1.2 Number of ligands per article.

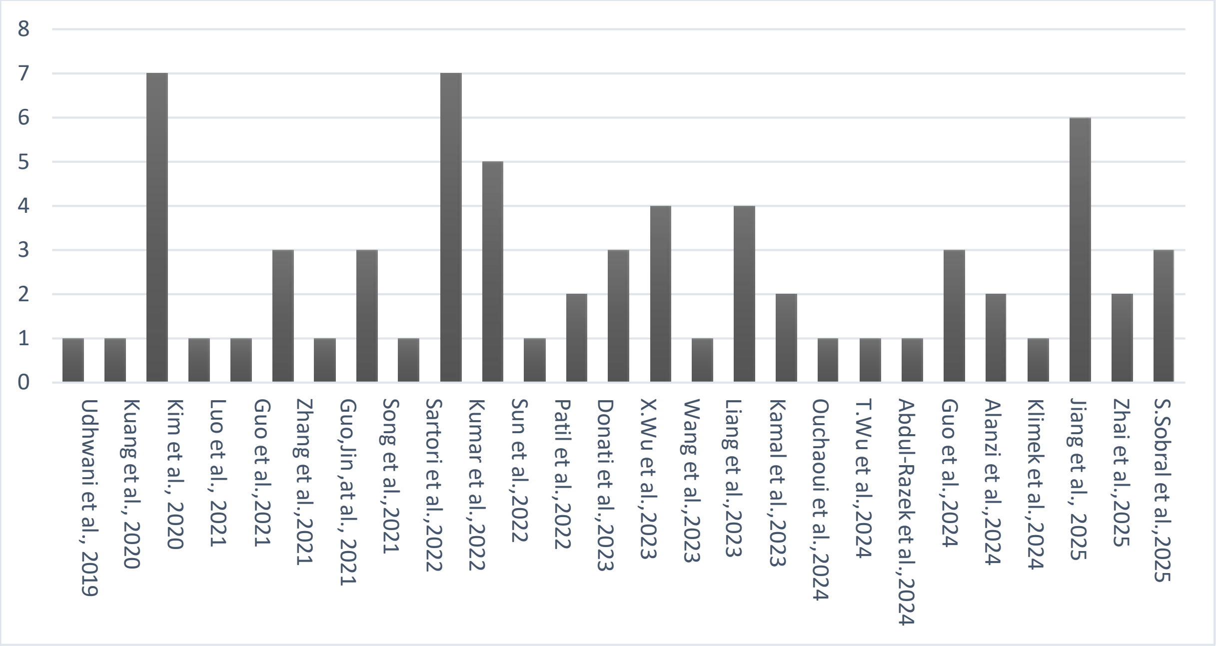


Figure 15: Bar chart representing ligand count per publication.

The selected articles differed in the number of ligands examined in each investigation. While some studies concentrated on a single PD-L1 inhibitor, others screened up to 7 ligands. This variation is a result of different research priorities; some studies sought to thoroughly describe a single lead chemical, while others screened structural analogues or natural product libraries. Overall, to provide a balanced representation in the meta-analysis, the variability in ligand counts per publication was considered during data extraction.

II.1.3 PDB ID Analysis

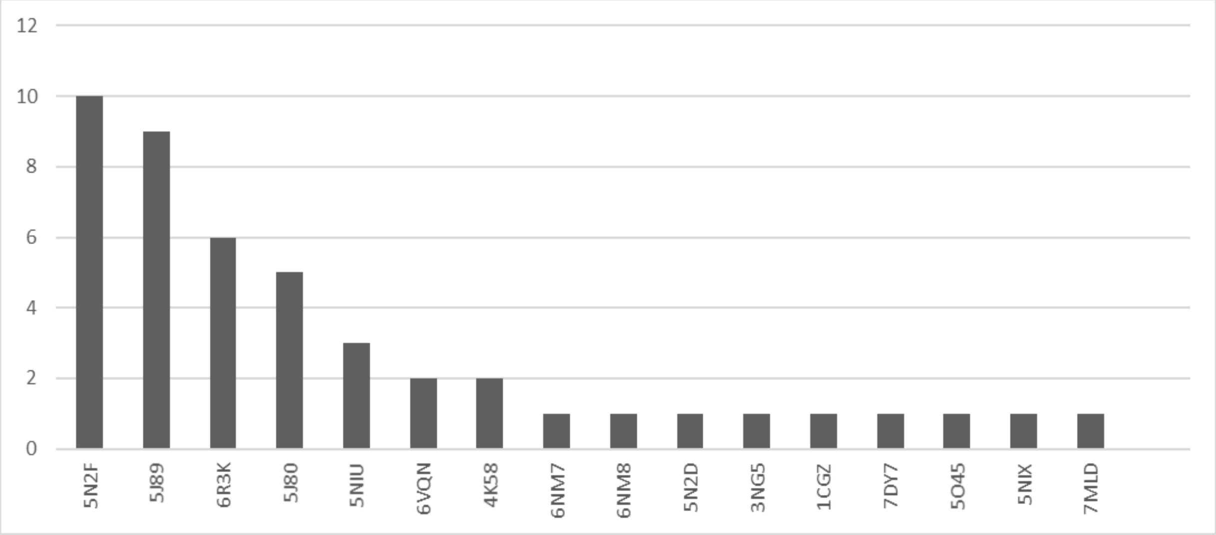
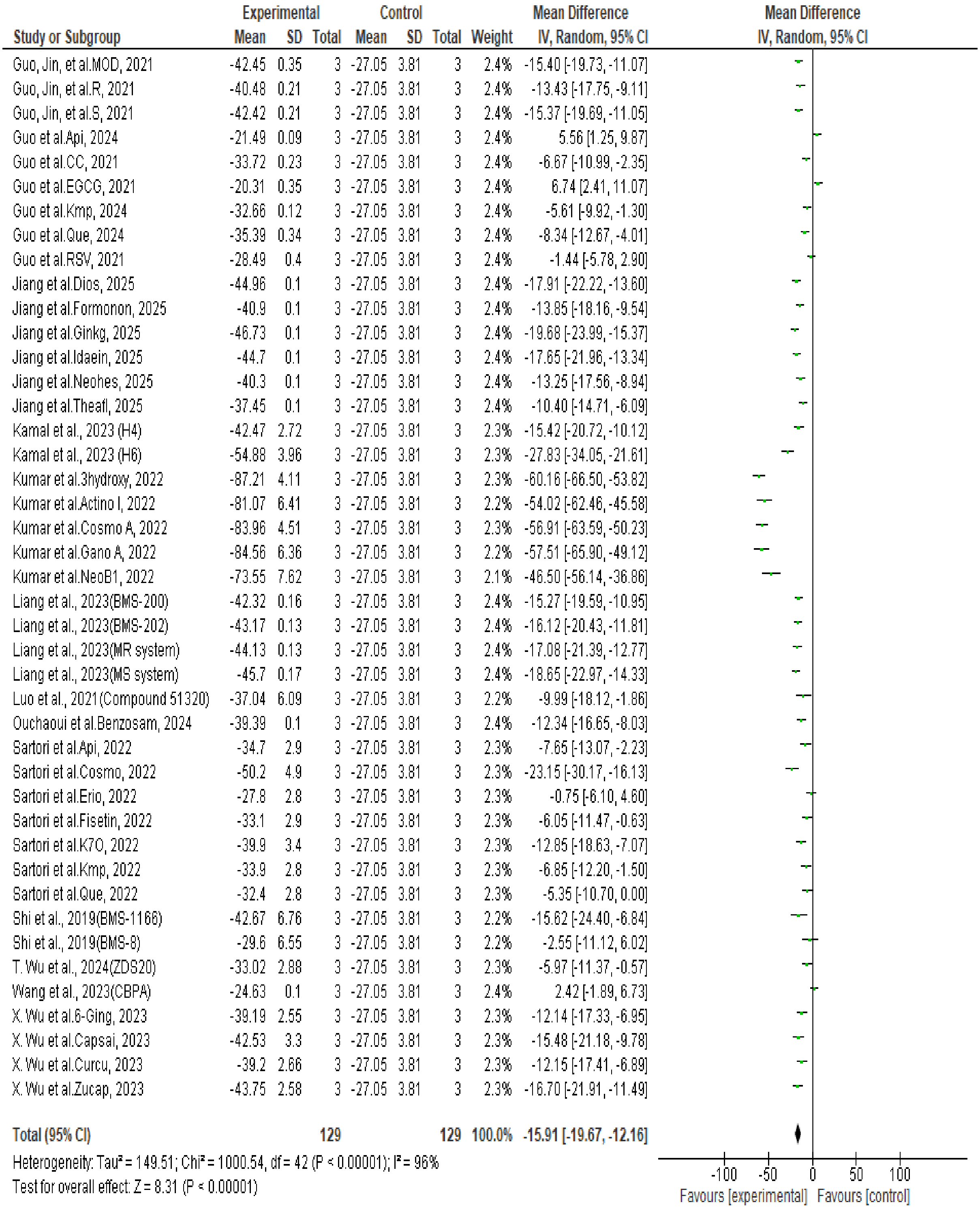


Figure 16: Frequencies of the different PDB IDs used across different studies.

All docking and simulation studies were based on PD-L1 structures and co-crystallized structures retrieved from the Protein Data Bank (PDB). Among the various PDB IDs used across the articles, PDB ID 5N2F was the most frequently used, which represents PD-L1 bound to a small molecule inhibitor. This structure appeared 10 times across different articles.

II.1.4 Binding Free Energy Analysis



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Guo, Jin, et al.MOD, 2021	-42.45	0.35	3	-27.05	3.81	3	2.4%	-15.40 [-19.73, -11.07]
Guo, Jin, et al.R, 2021	-40.48	0.21	3	-27.05	3.81	3	2.4%	-13.43 [-17.75, -9.11]
Guo, Jin, et al.S, 2021	-42.42	0.21	3	-27.05	3.81	3	2.4%	-15.37 [-19.69, -11.05]
Guo et al.Api, 2024	-21.49	0.09	3	-27.05	3.81	3	2.4%	5.56 [1.25, 9.87]
Guo et al.CC, 2021	-33.72	0.23	3	-27.05	3.81	3	2.4%	-6.67 [-10.99, -2.35]
Guo et al.EGCG, 2021	-20.31	0.35	3	-27.05	3.81	3	2.4%	6.74 [2.41, 11.07]
Guo et al.Kmp, 2024	-32.66	0.12	3	-27.05	3.81	3	2.4%	-5.61 [-9.92, -1.30]
Guo et al.Que, 2024	-35.39	0.34	3	-27.05	3.81	3	2.4%	-8.34 [-12.67, -4.01]
Guo et al.RSV, 2021	-28.49	0.4	3	-27.05	3.81	3	2.4%	-1.44 [-5.78, 2.90]
Jiang et al.Dios, 2025	-44.96	0.1	3	-27.05	3.81	3	2.4%	-17.91 [-22.22, -13.60]
Jiang et al.Formonon, 2025	-40.9	0.1	3	-27.05	3.81	3	2.4%	-13.85 [-18.16, -9.54]
Jiang et al.Ginkg, 2025	-46.73	0.1	3	-27.05	3.81	3	2.4%	-19.68 [-23.99, -15.37]
Jiang et al.Idaein, 2025	-44.7	0.1	3	-27.05	3.81	3	2.4%	-17.65 [-21.96, -13.34]
Jiang et al.Neohes, 2025	-40.3	0.1	3	-27.05	3.81	3	2.4%	-13.25 [-17.56, -8.94]
Jiang et al.Theafl, 2025	-37.45	0.1	3	-27.05	3.81	3	2.4%	-10.40 [-14.71, -6.09]
Kamal et al., 2023 (H4)	-42.47	2.72	3	-27.05	3.81	3	2.3%	-15.42 [-20.72, -10.12]
Kamal et al., 2023 (H6)	-54.88	3.96	3	-27.05	3.81	3	2.3%	-27.83 [-34.05, -21.61]
Kumar et al.3hydroxy, 2022	-87.21	4.11	3	-27.05	3.81	3	2.3%	-60.16 [-66.50, -53.82]
Kumar et al.Actino I, 2022	-81.07	6.41	3	-27.05	3.81	3	2.2%	-54.02 [-62.46, -45.58]
Kumar et al.Cosmo A, 2022	-83.96	4.51	3	-27.05	3.81	3	2.3%	-56.91 [-63.59, -50.23]
Kumar et al.Gano A, 2022	-84.56	6.36	3	-27.05	3.81	3	2.2%	-57.51 [-65.90, -49.12]
Kumar et al.NeoB1, 2022	-73.55	7.62	3	-27.05	3.81	3	2.1%	-46.50 [-56.14, -36.86]
Liang et al., 2023(BMS-200)	-42.32	0.16	3	-27.05	3.81	3	2.4%	-15.27 [-19.59, -10.95]
Liang et al., 2023(BMS-202)	-43.17	0.13	3	-27.05	3.81	3	2.4%	-16.12 [-20.43, -11.81]
Liang et al., 2023(MR system)	-44.13	0.13	3	-27.05	3.81	3	2.4%	-17.08 [-21.39, -12.77]
Liang et al., 2023(MS system)	-45.7	0.17	3	-27.05	3.81	3	2.4%	-18.65 [-22.97, -14.33]
Luo et al., 2021(Compound 51320)	-37.04	6.09	3	-27.05	3.81	3	2.2%	-9.99 [-18.12, -1.86]
Ouchaoui et al.Benzosam, 2024	-39.39	0.1	3	-27.05	3.81	3	2.4%	-12.34 [-16.65, -8.03]
Sartori et al.Api, 2022	-34.7	2.9	3	-27.05	3.81	3	2.3%	-7.65 [-13.07, -2.23]
Sartori et al.Cosmo, 2022	-50.2	4.9	3	-27.05	3.81	3	2.3%	-23.15 [-30.17, -16.13]
Sartori et al.Erio, 2022	-27.8	2.8	3	-27.05	3.81	3	2.3%	-0.75 [-6.10, 4.60]
Sartori et al.Fisetin, 2022	-33.1	2.9	3	-27.05	3.81	3	2.3%	-6.05 [-11.47, -0.63]
Sartori et al.K7O, 2022	-39.9	3.4	3	-27.05	3.81	3	2.3%	-12.85 [-18.63, -7.07]
Sartori et al.Kmp, 2022	-33.9	2.8	3	-27.05	3.81	3	2.3%	-6.85 [-12.20, -1.50]
Sartori et al.Que, 2022	-32.4	2.8	3	-27.05	3.81	3	2.3%	-5.35 [-10.70, 0.00]
Shi et al., 2019(BMS-1166)	-42.67	6.76	3	-27.05	3.81	3	2.2%	-15.62 [-24.40, -6.84]
Shi et al., 2019(BMS-8)	-29.6	6.55	3	-27.05	3.81	3	2.2%	-2.55 [-11.12, 6.02]
T. Wu et al., 2024(ZDS20)	-33.02	2.88	3	-27.05	3.81	3	2.3%	-5.97 [-11.37, -0.57]
Wang et al., 2023(CBPA)	-24.63	0.1	3	-27.05	3.81	3	2.4%	2.42 [-1.89, 6.73]
X. Wu et al.6-Ging, 2023	-39.19	2.55	3	-27.05	3.81	3	2.3%	-12.14 [-17.33, -6.95]
X. Wu et al.Capsai, 2023	-42.53	3.3	3	-27.05	3.81	3	2.3%	-15.48 [-21.18, -9.78]
X. Wu et al.Curcu, 2023	-39.2	2.66	3	-27.05	3.81	3	2.3%	-12.15 [-17.41, -6.89]
X. Wu et al.Zucap, 2023	-43.75	2.58	3	-27.05	3.81	3	2.3%	-16.70 [-21.91, -11.49]
Total (95% CI)			**129**			**129**	**100.0%**	**-15.91 [-19.67, -12.16]**

Heterogeneity: $Tau^2 = 149.51; Chi^2 = 1000.54, df = 42 (P < 0.00001); I^2 = 96\%$

Test for overall effect: $Z = 8.31 (P < 0.00001)$

Figure 17: Forest plot for binding free energies across studies, control BMS-202(107)

In this Meta-Analysis, we created a forest plot, comparing the binding free energies of different ligands from 15 of the 27 articles. These articles used the same computational calculations to obtain the binding free energies i.e. MM/GBSA (Molecular Mechanics with

Generalized Born and Surface Area solvation) or MM/PBSA (Molecular Mechanics Poisson-Boltzman Surface Area), as this method is more expensive but more accurate. Both of these methods provided the binding free energies in kcal/mol, allowing these energies to be compared among them. The other 12 articles provided binding affinities in K_d, IC_{50} or in form of docking scores that did not have units.

The aim for this forest plot was to see if the results from the research done are statistically significant. A set of observed data is said to be significant if it can be linked to a particular cause rather than being the product of chance. The binding free energies of 43 PD-L1 inhibitors were compared using a random effects model with BMS-202, from T. Wu et al., 2024 (107), as the reference compound. We assumed the total, n, as 3, as the articles did not mention the number of MM/GBSA or MM/PBSA runs performed. For articles that did not mention the standard deviation for the Binding Free Energies, we assumed the value 0.1.

In the forest plot above, we found that the observed data is statistically significant ($Z= 8.31$, $p < 0.00001$, 95% CI= [-19.67, -12.16].

II.1.5 RMSD Analysis

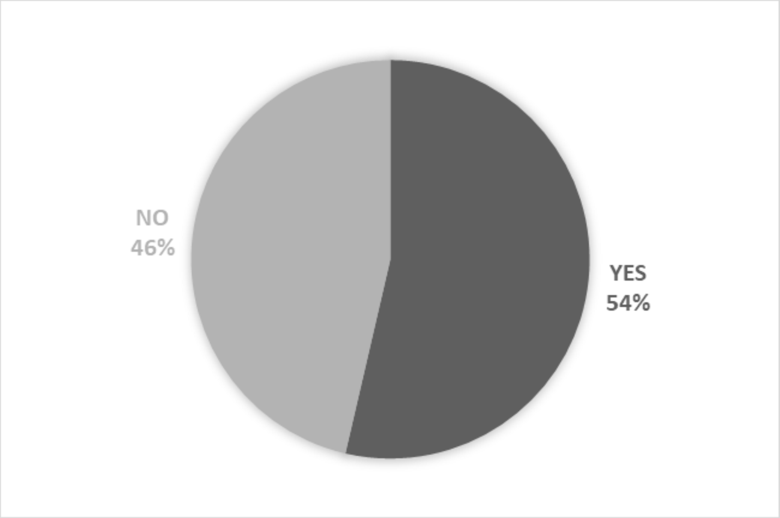


Figure 18: Pie chart representing the presence or absence for RMSD in articles.

To evaluate the structural stability of the protein-ligand complexes, Root Mean Square Deviation (RMSD) values were collected from Molecular Dynamics Simulations reported across the included studies. The RMSD values reflects the fluctuation of the ligand-protein complex over time, including the binding stability and conformational changes of the ligand within the PD-L1 binding site. The pie chart above shows that only 37 of the 69 ligands reported RMSDs, representing 54% of the total ligands.

- RMSD < 2 Å: Highly stable complexes with minimal deviation from the initial docking pose.
- RMSD between 2.0 - 3.0 Å: moderate fluctuations, often associated with flexible ligands or loop movement in the PD-L1 structure.
- RMSD > 3.0 Å: Suggests notable instability or weak anchoring within the binding site.

The lowest RMSD value was 0.40 Å, molecule X superposed on BMS-8 from **Kim et al., 2020**(106)**.** The second lowest value was an RMSD value between 0.5–1.5 Å for molecule ZDS20 from **T. Wu et al., 2024**(105)**.**

II.1.6 Recurring Amino Acids

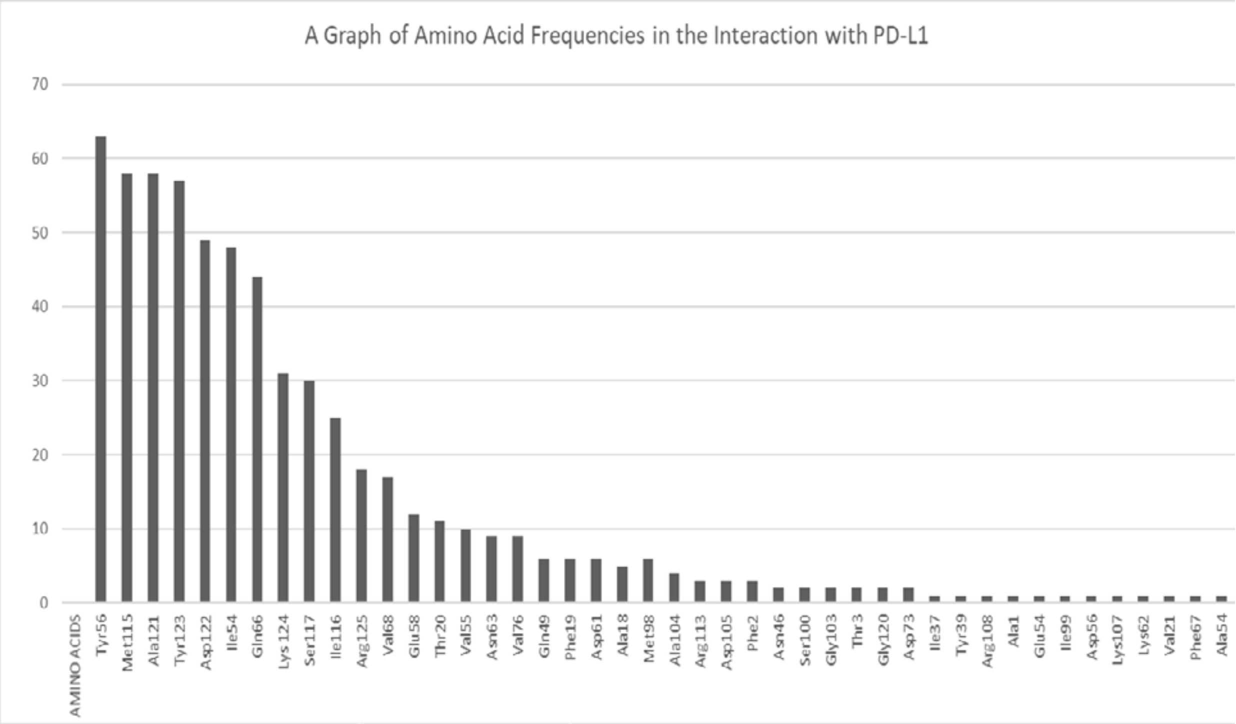


Figure 19: Bar Chart of Amino Acid Frequencies in the Interaction with PD-L1.

To identify the common key residues across ligands, amino acids involved in binding with PD-L1 were extracted from all included molecular docking and simulation studies. The residues were catalogued and the frequency of each amino acid's involvement was quantified.

A total of 44 amino acids were found to interact with the studied inhibitors. Among these Tyr56, Met115 and Ala121 were the top 3 most recurrent, appearing 63, 58 and 58 times respectively. These residues were involved in different types of interactions, suggesting they have a key role in ligand stabilization within the PD-L1 binding groove. **(Fig. 18)**

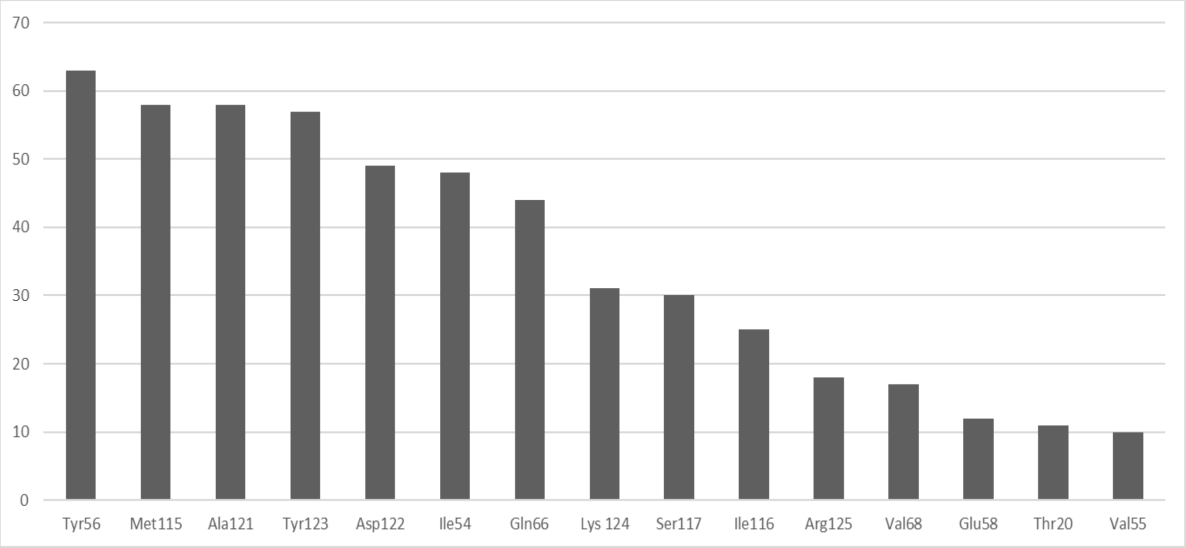


Figure 20: Top residues in the interaction with PD-L1.

Another bar chart of residues with the highest frequencies (top residues) was created to clearly depict the amino acids that were important in the interaction with PD-L1. This suggests that future PD-L1 inhibitor design may benefit from targeting an interaction pattern involving these key residues. **(Fig.19)**

II.1.7 Molecular Interactions.

Table 1: Table of Molecular Interactions of PD-L1 inhibitors across different articles

Article	Ligand	Bonds	Amino acids
1. Abdulrazek et al.	Que-SNPs	3 H-bonds	Phe19, Asp122, Tyr123
		Water mediated H-Bond	Gln66, Asp122
2. Alanzi et al.	a) 49642753	π-Sigma	Ala121, Met115
		Alkyl	Tyr56, Ile54, Met115
		Halogen	Asp122, Ala121
	b) 857389	π-Sigma	Ala121
		Alkyl	Tyr56, Met115, Ala121
3. Donati et al.	a) BMS 202	π-π	Tyr56
		Water mediated interaction	Asp122, Lys124
		induced π-π	Tyr123
		induced H-bond	Glu58
		another induced H-bond	Arg113
		Electrostatic interaction	Asp122
		Labile H-bond	Lys124
		π-sulfur	Met115
	b) RS39	π-π	Tyr56
		Water mediated interaction	Asp122, Lys124
		H-bond	Gln66
		induced π- π	Tyr123
		induced H-bond	Glu58
		Another induced H-bond	Arg113
		π-sulfur	Met115
	c) P18	H-bonds	Gln66, Arg125
		π-π	Tyr56, Tyr123
		Electrostatic interaction	Asp73, Asp122
		induced H-bond	Glu58
		π-sulfur	Met115

4. Guo et al., 2021	a) S-BMS200	H-bond	Phe19
		Hydrophobic interaction	Phe19, Ile54, Tyr56, Met115, Ala121, Tyr123, Ala66, Val68
	b) R-BMS200	H-bond	Asp122, Lys124
		Hydrophobic interaction	Ile54, Tyr56, Met115, Ala121, Asp122, Tyr123, Lys124, Val68
5. Guo et al., 2021	a) BMS-200	H-bonds	Gln66, Asp122
		π-π	Tyr56
		Hydrophobic interaction	Met115, Ala121, Tyr123, Ile54, Tyr56
	b) CC	H-bonds	Gln66, Ser117, Asp122
		π-π	Tyr56
		Hydrophobic interaction	Met115, Ala121, Ile54, Val68
	c) RSV	H-bonds	Tyr56, Met115, Tyr123
		Hydrophobic interaction	Met115, Ala121, Ile54, Val68
	c) EGCG	H-bonds	Phe19, Thr20, Ala121, Lys124, Gln66, Met115, Asp122
		Hydrophobic interaction	Met115, Ala121, Ile54, Val68
6. Guo et al., 2024	a) Api	H-bond	Lys124
	b) Kmp	H-bond	Asp122, Ala121, Ile116, Ile54, Met115
	c) Que	H-bond	Gln66, Ile116, Ile54, Asp122, Tyr123
7. Jiang et al., 2025	a) Ginkgetin	H-bond	Arg108, Asn172, Gln175, Asp231
		Van der Waals	Ile99, Ser100, Val38, Ile225, Ile163

		C-H bonds	Ile37, Met98, Ala230, Ser226, Val177
		π-Cation	Asp105
		π-Sulfur	Met224
		π-alkyl	Ala104
		Alkyl	Tyr39, Val185
	b) Theaflavin	H-bond	Gln175, Ser226, Met224, Asn172
		C-H bonds	Gly103, Ala1, Ala104
		Van der Waals	Val164, Phe2, Thr3, Val185, Tyr106, Ser100, Met98, Ala230, Hie178
		π-ion	Asp105
		π-π	Tyr165
		π-alkyl	Ile163, Val177
	c) Diosmin	H-bond	Phe2, Thr3, Asp105, Glu180
		C-H bonds	Ala104, Asp231
		Van der Waals	Ile225, Met224, Ser226, Ile163, Val164, Phe176, Val177, Glu181, Asp182, Lys107, Ile99, Ser100, Ile37, Tyr232, Gln175, Tyr106
		π-sulfur	Met98
		Alkyl	Ala1, Ala230, Tyr39
		π-π	Tyr165
	d) Formononetin	H-bond	Ile225
		C-H bonds	Gln175, Ile99, Met224
		Van der Waals	Val38, Ser100, Asp105, Tyr106, Tyr165, Phe176, Val177, Asn172, Val164,

			Ser226, Tyr232, Asp231, Val51, Tyr39
		π-alkyl	Ala104, Ala230, Met98, Ile37
		Alkyl	Ile163
	e) Idaein	H-bond	Met224, Asp105, Ala104, Gln175
		C-H bonds	Phe2, Gly103
		Van der Waals	Phe176, Val164, Ser226, Ile225, Ile99, Met98, Hie178, Val177, Ala1, Thr3, Lys107
		π-π	Tyr165, Tyr106
		π-alkyl	Ile163, Val185
	f) Neohesperidine	H-bond	Phe2, Asp182, Gln175, Ala104, Lys107, Tyr165
		C-H bonds	Thr3
		Van der Waals	Lys184, Ala1, Val77, Ile225, Ser226, Ser100, Ile99, Met224, Tyr106, Arg96, Glu167
		π-sulfur	Met98
		π-ion	Asp105
8. Kamal et al.,2023	a) H6	H-bond	Asp122, Gln66
	b) H4	H-bond	Ala121, Met115, Gln66
9. Kim et al.,2020	a)BMS8	H-bond	Gln66
		Hydrophobic interaction	Val68
	b) X	H-bond	Tyr56
10. Klimek et al.,2024	7j	H-bond	Tyr56, Asp61, Lys124, Arg125
		π-π	Tyr56
	BMS inhibitors	H-bond	Ala121

11. Kuang et al., 2022		Hydrophobic interaction	Tyr56, Glu58, Arg113, Met115, Tyr123
12. Kumar et al.,2022	a) Neonactin B1	H-bond	Tyr123, Lys124, Tyr56, Asp61
		Hydrophobic interaction	Ile54, Tyr56, Met115, Ile116, Ala121, Tyr123
		Polar interaction	Ser117, Asn63, Gln66
		Salt bridge	Asp61
	b) Actinofuranone I	H-bond	Asp122, Tyr56, Asn63
		Hydrophobic interaction	Ala18, Phe19, Ile54, Val55, Tyr56, Met115, Ile116, Ala121, Tyr123, Val68, Val76
		Polar interaction	Thr20, Gln66, Ser117, Asn63
	c) Cosmosporin A	H-bond	Asp122, Tyr123, Lys124
		Hydrophobic interaction	Ile54, Tyr56, Met115, Ile116, Ala121, Tyr123
		Polar interaction	Ser117, Gln66
	d) Ganocapenoid A	H-bond	Ala121
		Hydrophobic interaction	Ile54, Tyr56, Val68, Met115, Ile116, Ala121, Tyr123
		Polar interaction	Gln66, Ser117
		π-π	Tyr56
	e) 3-[3-hydroxy…]	H-bond	Ala121, Met115
		Hydrophobic interaction	Ile54, Tyr56, Met115, Ile116, Ala121, Tyr123, Val55
		Polar interaction	Gln66, Ser117
		π-π	Tyr56
13. Liang et al.,2023	a) BMS200	H-Bond	Asp73, His69
		π-π	Tyr56

	b) BMS202	H-Bond	Gln66, Asn63
		π-π	Tyr56
	c) MS system	H-bond	Gln66
		π-π	Tyr56
	d) MR system	H-bond	Gln66, Tyr56
		π-π	Tyr56
14. Luo et al., 2021	compound 51320	H-bond	Ala121
		Ionic Interaction	Asp122
		π-π	Ile54
		π-sigma	Tyr123
15. Ouchaoui et al., 2024	Benzosampangine	Hydrophobic interaction	Tyr56, Met115, Ala121, Tyr123
		H-bond	Ile116, Ile54
		C-H bond	Ser117
		Water bridge	Arg113
		π-Sulfur	Met115
		π-π	Tyr56
		π-alkyl	Ala121
		Van der Waals	Ile54, Gln66, Ile116, Asp122, Tyr123
16. Patil et al., 2022	a) CRT5	H-bond	Asn63, Ala121, Arg125
		Hydrophobic interaction	Ala121, Tyr123, Ile54, Tyr56, Val76, Met115
		Ionic Interaction	Arg125
		π-π	Tyr56
		Water bridge	Phe19, Ala121, Asp122, Lys124, Arg125, Asp61, Asn63, His69, Val76
	b) P053	H-bond	Ala121, Asp122, Gln66
		Hydrophobic interaction	Ile54, Tyr56, Met115, Ala121, Tyr123, Val68

		Water bridge	Phe19, Thr20, Ala121, Asp122, Tyr123, Gln66, Asp73
17. S. Sobral et al., 2025	a) BMS 200	Hydrophobic interaction	Ile54, Tyr56, Met115, Ala121, Tyr123
		H-bond	Lys124
		π-π	Tyr56
	b) Sonidegib	Hydrophobic interaction	Ile54, Tyr56, Met115, Ala121, Tyr123
		H-bond	Gln66, Ala121
	c) Lapatinib	Hydrophobic interaction	Tyr56, Met115, Ala121, Tyr123
		H-bond	Asn63, Gln66
		π-π	Tyr123
18. Sartori et al. 2022	a) Cosmosiin	H-bond	Ile116, Glu58, Lys124, Tyr123, Asp122, Asp61, Gln66, Tyr56, Tyr118
		π-π	Tyr56
		Salt bridge	Glu61, Arg125
		Water mediated H-Bond	Asp61, Glu58
	b) Apigenin	Hydrophobic interaction	Met115, Ala121, Ile54
		H-bond	Gln66, Ser117
		Water mediated H-Bond	Gln66, Asp122
		Water bridge	Ala121
		π-π	Tyr56
	c) Kmp	π-π	Tyr56
		H-bond	Ile116, Ala121
		Water bridge	Gln66, Asp122
		Hydrophobic interaction	Tyr56, Val68, Ile54
		Water mediated H-Bond	Asp73
	d) K7O	H-bond	Tyr56, Glu58, Asn63, Asp61, Tyr123
		π- π	Tyr56

	e) Quercetin	Water bridge	Gln66, Asp122
	f) Fisetin	H-bond	Ile116, Gln66, Asp122
		π-π	Tyr56
		Water bridge	Gln66, Asp122, Ala121
	g) Eriodyctiol	Hydrophobic interaction	Met115, Ile54, Ala121, Tyr123
		H-bond	Ile116
		Water bridge	Gln66, Asp61
19. Shi et al., 2019	a) BMS8	H-Bond	Gln66
		Water bridge	Lys124, Gln66, Tyr123, Asp122
		Halogen	Ile54
	b) BMS1166	H-Bond	Asp122
		Water bridge	Lys124, Thr20, Arg125, Gln66, Lys75, Val76, Asp122, Phe19, Ala121, Tyr123
		Halogen	Ile54
20. Song et al., 2021	a) BMS202	π-π	Tyr56
		π-carbonyl	Ala121
	b) compound 43	H-bonds	Asp122, Lys124, Gln66
		π-π	Tyr56
		Electrostatic interaction	Asp122
21. Sun et al., 2022	P39	H-bond	Asp122, Lys124, Tyr56
		π-π	Tyr56
		π-sigma	Ala121
		π-alkyl	Met115
		π-Cation	Arg125
22. Udhwani et al., 2019	XGIQBUNWFCCM….	H-bond	Phe19, Asp122
		Van der Waals	Val55, Asp122, Ile54, Ala125, Ser117, Ile116, Phe67, Val68, Ala18, Gly120, Tyr56, Tyr123

		Electrostatic interaction	Gln66, Met115, Asp122, Ala121, Thr20, Phe19
		π- π	Tyr56
		π-sigma	Asp122
23. Wang et al., 2023	CBPA	Hydrophobic interaction	Ile54, Val55, Tyr56, Val68, Met115, Ile116, Ala121, Tyr123
		H-bond	Gln66
24. Wu et al., 2024	ZDS20	Hydrophobic interaction	Tyr56, Met115, Ile54, Ala121, Tyr123
		π-π	Tyr56,Tyr123
		π-Cation	Lys124
		H-bond	Tyr56, Tyr123
25. Wu et al., 2023	a) Capsaicin	H-bond	Gln66
		Hydrophobic interaction	Ile54, Tyr56, Gln66, Met115, Ala121, Tyr123
	b) Zucapsaicin	H-bond	Gln66
		Hydrophobic interaction	Tyr56, Gln66, Met115, Ala121, Tyr123, Ile54
	c) 6- Gingerol	H-bond	Gln66, Ser117
		Hydrophobic interaction	Ile54, Tyr56, Val68, Met115, Ala121, Tyr123
	d) Curcumin	H-bond	Tyr123
		Hydrophobic interaction	Met115, Ala121, Tyr123, Ile54, Tyr56, Gln66
26. Zhai et al., 2025	a) 58	H-bond	Ile116, Tyr123, Phe67, Val55
		π-π	Tyr56
	b) 34	H-bond	Ala121, Phe67, Val68
		π-π	Tyr56
27. Zhang et al., 2021	A9	π-π	Tyr56
		Ionic Interaction	Asp122
		H-bond	Tyr123, Tyr56, Arg125

To better understand the binding mechanisms of PD-L1 inhibitors, molecular interactions were analyzed based on docking studies and molecular dynamics simulations from selected studies. Interactions were categorized into Hydrogen bonds, Hydrophobic contacts (alkyl, pi-alkyl, Van der Waals), electrostatic interactions (salt bridges, pi-cation, pi-anion), pi-related interactions (pi-pi stacking, T-stacking, pi-sulfur), water-bridged H-bonds, and halogen or polar interactions. The data was formulated in the form of a table as seen above.

A comparative summary of interaction types and residues for each compound revealed that effective PD-L1 inhibition relies on a combination of Hydrogen bonding, Hydrophobic interactions and Pi-interactions, particularly within the PD-L1 dimer interface. (Table 1)

Table 2 : Substituents involved in Hydrogen Bonding with PD-L1 dimer.

Study	Substituents involved in H-bonds	
	Donors	Acceptors
1. Zhang et al.,2021	(NH) amine	(C=O) carbonyl (amide) R-O-R (ether) F (weak acceptor)
2. Zhai et al.,2025	(NH) amine (OH) hydroxyl	(C=O) carbonyl (carboxylique)
3. X.Wu et al.,2023	(NH) amide (OH) hydroxyl	(C=O) carbonyl (amide)
4. T.Wu et al.,2024	(NH) amine	(C=O) carbonyl (amide)
5. Wang et al.,2023	(NH) amine	(-)
6. Udhwani et al., 2019	(NH) amine (NH) amide	(-)
7. Sun et al.,2022	(NH) amine (OH) hydroxyl	(C=O) carbonyl (carboxylique)
8. Song et al.,2021	(NH) amine (OH)hydroxyl (D-mannosamine)	(C≡N) nitrile
9. Shi et al., 2019	(NH) amine	(C=O) carbonyl (C≡N) nitrile R-O-R (ether)
10. Sartori et al.,2022	(OH) hydroxyl	(C=O) carbonyl

11. S.Sobral et al.,2025	Imidazole	Pyridine
	(NH) amide	
12. Patil et al.,2022	(NH) amide	Pyridine
	(NH2) amine	
13. Ouchaoui et al.,2024	(-)	Pyridine
14. Luo et al., 2021	(NH) amide	(-)
	(NH) amine	
15. Liang et al.,2023	(OH) hydroxyl	(C=O) carbonyl (amide)
	(NH) amine	Pyridine
16. Kumar et al.,2022	(OH) hydroxyl	(C=O) carbonyl
	(NH3+) ammonium	
17. Kuang et al., 2020	aromatic (NH)	(C=O) carbonyl (amide)
	(NH) amine	(C=O) carbonyl (carboxylique)
	(NH) amide	R-O-R (ether)
	(OH) hydroxyl	
18. Klimek et al.,2024	(NH) amide	(C=O) carbonyl (amide)
19. Kim et al., 2020	(NH2) amine	(-)
20. Kamal et al.,2023	(NH) amine	(C=O) carbonyl (amide)
	(NH) amide	
	(OH) hydroxyl	
	Hydrazone	
	Thiourea	
21. Jiang et al., 2025	(OH) hydroxyl	(C=O) carbonyl
		R-O-R (ether)
22. Guo et al.,2024	(OH) hydroxyl	phenol
23. Guo et al.,2021	(OH) hydroxyl	(C=O) carbonyl
24. Guo, Jin at al., 2021		(C=O) carbonyl
		R-O-R (ether)
25. Donati et al.,2023	(NH) amide	(C=O) carbonyl (amide)
	(NH) amine	(C≡N) nitrile
	(NH2) amine	
26. Alanzi et al.,2024	(-)	(-)
27. Abdul-Razek et al.,2024	(OH) hydroxyl	(-)

Since Hydrogen Bonds were identified as the most frequent interaction, we analyzed the substituents from different ligands across the included studies and formulated a comparative table, summarizing the H-bonds across the studies. We classified different substituents as either donors or acceptors to clearly understand the hydrogen bonding within the PD-L1 dimer. (Table 2)

Table 3: Substituents involved in π-π stacking

STUDY	SUBSTITUENTS INVOLVED IN π-π STACKING
1. Zhang et al.,2021	Fluorophenyl
	Pyridine
2. Zhai et al.,2025	Benzene
3. X.Wu et al.,2023	(-)
4. T.Wu et al.,2024	Phenyl
5. Wang et al.,2023	(-)
6. Udhwani et al., 2019	Pyridine
7. Sun et al.,2022	Pthalimide
	Benzene
8. Song et al.,2021	Phenyl
	(1 F) from Difluoromethyleneoxy
9. Shi et al., 2019	Biphenyl
10. Sartori et al.,2022	Chromen-4-one
11. S.Sobral et al.,2025	Furan
12. Patil et al.,2022	Phenyl
13. Ouchaoui et al.,2024	2 pyridine rings
	1 benzene ring
14. Luo et al., 2021	Phenyl
15. Liang et al.,2023	Biphenyl moiety
16. Kumar et al.,2022	Benzene
	Phenol
17. Kuang et al., 2020	Biphenyl
	Phenyl
18. Klimek et al.,2024	bromophenyl
	phenyl
19. Kim et al., 2020	(-)

20. Kamal et al.,2023	(-)
21. Jiang et al., 2025	Benzopyrilium
	Flavone
	Chromenone
22. Guo et al.,2024	Flavone
23. Guo et al.,2021	Phenyl
24. Guo, Jin ,at al., 2021	(-)
25. Donati et al.,2023	Biphenyl
	Pyridine
	Triazine
	Chlorophenyl
26. Alanzi et al.,2024	(-)
27. Abdul-Razek et al.,2024	(-)

Several high affinity PD-L1 inhibitors demonstrated π-π stacking interactions. These are non-covalent contacts between aromatic systems through delocalized π-electrons. These interactions, often observed in ligands with phenyl or heteroatomic groups such as chromen-4-one, flavone and benzo-fused rings, played a significant role in complex stabilization. The frequency of these interactions highlights how crucial aromatic moieties are in future PD-L1 inhibitor design. (Table 3)

Table 4: Substituents involved in other π interactions.

Study	**Other π interactions**
1. Alanzi et al.,2024	π-alkyl (phenyl)
2. Donati et al.,2023	π-sulfur (biphenyl)
3. Jiang et al., 2025	π-sulfur (phenyl)
	π-alkyl (chromenone)
	π-alkyl (phenyl)
4. Luo et al., 2021	π-sigma (phenyl)
5. Ouchaoui et al.,2024	π-sulfur (pyridine, tetralone)
	π-alkyl (phenyl)
6. Sun et al.,2022	π-sigma (biphenyl)
	π-alkyl (biphenyl)

Besides π-π stacking, the other π interactions were π-sulfur contacts with residues like Met115, π-alkyl with aliphatic side chains like that of Ala121 and occasional π-sigma interactions. Though weaker overall, these interactions contributed to the general binding affinity and positioning of the ligand into the PD-L1 pocket. (Table 4)

Table 5 : Substituents involved in Hydrophobic interactions.

Study	**Substituents involved in Hydrophobic interactions**
1. Zhang et al.,2021	Fluorophenyl (moderate-strong)
	Toluene (very strong)
	Methoxy (weak)
	Pyridine (moderate)
2. Zhai et al.,2025	Chlorophenyl (strong-moderate)
	Benzodioxan (strong-moderate)
	Thiazole (moderate)
	Pyrazole (moderate)
3. X.Wu et al.,2023	Isopropyl (moderate-strong)
	Methoxy (weak)
	Pently (very strong)
	Phenyl (very strong)
4. T.Wu et al.,2024	Tetraisoquinolone (moderate)
	Phenyl (very strong)
5. Wang et al.,2023	Pyridine (moderate)
	Chlorophenyl (strong- moderate)
	Anisole(methoxyphenyl) (moderate)
6. Udhwani et al., 2019	Methoxy (weak)
	Methyl (weak)
	Biphenyl (very strong)
	Pyridine (moderate)
7. Sun et al.,2022	Biphenyl (very strong)
	Phenyl (very strong)
8. Song et al.,2021	Difluoromethylenoxy (strong-moderate)
	Chloro (moderate)
	Methyl (weak)
	Benzopyran (moderate)

9. Shi et al., 2019	Benzodioxan (strong-moderate)
	Chlorophenyl (strong-moderate)
	Bromophenyl (strong-moderate)
	Biphenyl (very strong)
	Methyl (weak)
10. Sartori et al.,2022	Phenyl (very strong)
	Flavone core (strong-moderate)
11. S.Sobral et al.,2025	Dichlorophenyl (strong-moderate)
	Methoxy (weak)
	Toluene (very strong)
	Fluorophenyl (strong-moderate)
	Chlorophenyl (strong-moderate)
	Furan (moderate)
	Benzodioxan (strong-moderate)
	Trifluoromethyl (strong-moderate)
	Methyl (weak)
12. Patil et al.,2022	Dichlorophenyl (strong-moderate)
	Methoxy (weak)
	Phenyl (very strong)
	Methyl (weak)
13. Ouchaoui et al.,2024	Tetralone (benzene, cyclohexane) (strong-moderate)
	Benzene, 2 pyridine (tricycle) (strong-moderate)
14. Luo et al., 2021	Methyl (weak)
	Phenyl (very strong)
15. Liang et al.,2023	Methoxy (weak)
	Biphenyl (very strong)
	Toluene (very strong)
	Benzodioxan (strong-moderate)
16. Kumar et al.,2022	Hexyl (very strong)
	Ethyl (moderate)
	Isopropyl (strong-moderate)
17. Kuang et al., 2020	Methoxy (weak)
	Bromophenyl (strong-moderate)

	Biphenyl (very strong)
	Toluene (very strong)
	Difluorophenyl (strong-moderate)
	Benzodioxan (strong-moderate)
	Chlorophenyl (strong-moderate)
18. Klimek et al.,2024	Toluene (very strong)
	Methoxy (weak)
	Bromophenyl (strong-moderate)
19. Kim et al., 2020	Bromophenyl (strong-moderate)
	Methoxy (weak)
20. Kamal et al.,2023	Indole (strong-moderate)
	Isopropyl (strong-moderate)
	Quinoline (mild-moderate)
	Phenyl (very strong)
21. Jiang et al., 2025	Phenyl (very strong)
	Methyl (weak)
	Methoxy (weak)
22. Guo et al.,2024	Phenyl (very strong)
23. Guo et al.,2021	Benzopyran (strong-moderate)
	Phenyl (very strong)
	Methoxy (weak)
24. Guo, Jin at al., 2021	Difluorophenyl (strong-moderate)
	Toluene (very strong)
	Benzodioxan (strong-moderate)
	Methoxy (weak)
25. Donati et al.,2023	Chlorophenyl (strong-moderate)
	Biphenyl (very strong)
26. Alanzi et al.,2024	Toluene (very strong)
	Trifluoromethyl (strong-moderate)
	Phenyl (very strong)
27. Abdul-Razek et al.,2024	Cyclohexyl (very strong)
	Phenyl (very strong)
	Methyl (weak)

Hydrophobic interactions were prevalent between the non-polar components of the ligands and hydrophobic residues at the PD-L1 binding site, namely Ala121, Val68, Ile115 and Ile54. These substituents such as alkyl chains, isopropyl groups, phenyl rings without polar groups, biphenyl and other non-polar groups. Even chromenone, benzodioxan and ethyleneoxy cores were significant for hydrophobic interactions. These non-directional but stabilizing contacts were all part of contributing to ligand residence time and general binding stability, complementing directed forces such as hydrogen bonding and π-π interactions. The widespread involvement of these groups highlights their potential as valuable structural motifs for further ligand optimization. (Table 5.)

II.2 Discussion

The goal of this meta-analysis was to compile information from various computational studies exploring the interaction of small molecules with PD-L1, an immune checkpoint protein linked to tumor immune evasion. The temporal analysis of publications revealed a progressive increase in the number of articles related to PD-L1 inhibitors over recent years. This reflects a growing interest in PD-L1 inhibitor research, with more articles after 2019. This upward trend is consistent with the advancement of immuno-oncology in general and the growing availability of in silico drug design techniques.

Furthermore, the number of ligands assessed per article varied greatly, indicating different research priorities. Some studies deeply characterized one or two molecules, while others opted for a larger virtual screen. This disparity implies the necessity for uniform evaluation standards in computational studies on inhibitors and probably adds to heterogeneity in the meta-analysis.

Among the chosen studies, PDB ID analysis showed that 5N2F was the most commonly used structure. It had an outstanding resolution of 1.70 Å, the highest quality structure among those included. It provides a detailed view of the binding interface and dimeric arrangement of PD-L1, making it a preferred template for molecular docking and interaction profiling. This inclination towards high resolution structures such as 5N2F suggests that researchers are working together to guarantee precise modeling of ligand-target interactions.

Our meta-analysis of binding free energies using a random-effects model with BMS-202, (T. Wu et al., 2024), as the reference compound demonstrated a statistically significant overall effect ($Z= 8.31$, $p < 0.00001$, 95% CI= [-19.67, -12.16]. Since the Z- score was above 1.96 (8.31) and the p-value was less than 0.05 (< 0.0001) we could rule out the null hypothesis and that there is a significant difference in the binding energies and that it is not by chance. Moreover, the analysis revealed a high level of heterogeneity of 96% ($I^2 > 75\%$), pointing to substantial variability among the included studies While an acceptable range typically falls between 25% and 50%, such elevated heterogeneity is not uncommon in computational meta-analyses and may result from differences in ligand structures, simulation protocols, docking settings, or binding energy calculation methods such as MM/GBSA and MM/PBSA. Despite this, the use of a random-effects model appropriately accounts for this variability, allowing for meaningful interpretation of the overall effect. (fig.16)

As for pose reliability and docking accuracy, RMSD values were provided for 54% of the ligands (37 of the 69 ligands). Among those, a good percentage of them achieved RMSD values of ≤ 2 Å, which would typically indicate high stability and conformational similarity to the target pose. The lowest value was 0.40 Å, molecule X superposed on BMS-8 from **Kim et al., 2020** (108). The second lowest was between 0.5-1.5 Å for molecule ZDS20 from **T. Wu et al., 2024** (107). However, the majority of the ligands fell in the range of 2-3 Å, which would represent good but more flexible binding performances. A smaller group of structures existed between 3-4 Å, which indicated greater conformational diversity and perhaps deviation from best docking positions. Notably, one outlier exhibited an abnormally high RMSD of 12.9 Å, Kaempferol from **Sartori et al.,2022** (109), which questions the validity or stability of its predicted binding conformation. This difference in RMSD values points to the importance of considering structural consistency alongside binding energy, as compounds with favorable energies may not necessarily have stable or well defined docking conformations.

This study also delved into molecular determinants of binding by analyzing recurring amino acids. Residues such as Tyr56, Met115, Ala121 and Tyr123 emerged consistently across high-affinity compounds. These residues correspond those identified as playing critical roles in the formation of the PD-1/PD-L1 interface by **Fantacuzzi et al., 2024** (95**).**

A thorough analysis of molecular interactions showed the ligand-PD-L1 complexes were stabilized by a variety of non-covalent bonds. Among the 69 ligands analyzed, Hydrogen bonds were the most frequent and contributed significantly to binding affinity and specificity. Common residues for H-bonds included Asp122, Ala121 and Gln66. Hydrophobic interactions, particularly with Met115, Ile54 and Tyr123, also played a key role in stabilization of the ligand within the PD-L1 pocket. π-π stacking interactions, especially involving Tyr56 and Tyr123, were also prevalent among compounds that had aromatic rings. An exception was noticed in the food derived compounds studied by **Jiang et al.,2025** (110), where π-π stacking mostly involved Tyr165. Water mediated H-bonds, often involving Asp122 and Gln66, further enhanced binding stability through indirect interaction networks. Less frequent but notable interactions were π-cation, π-anion (electrostatic) and π-sulfur interactions, mainly in ligands with charged or aromatic groups. The presence of labile or transient H-bonds, sometimes forming after the loss of π-stacking, as seen with BMS-202 and its analogues, indicates dynamic binding that may be relevant to biological activity. This is seen in **Donati et al.,2023.** (111)

A close examination of the ligands provided a common pattern of structural substituents that made significant contributions to binding through Hydrogen bonding, π-π stacking and other π interactions, in addition to hydrophobic contacts. Common donors of hydrogen bonds included hydroxyl (-OH) and amine (-NH-) groups. Hydrogen bond acceptors included carbonyl containing substituents (-C=O) and nitriles (-C≡N). For π-π stacking and other π interactions such as π-sulfur and π-alkyl, key contributors were phenyl rings, fused aromatic systems such as flavones (109), chromenone cores (110), and polycyclic structures like benzosampangine (112), constructed from benzene, pyridine and acridine moieties. These systems enable extensive planar stacking with aromatic residues such as Tyr56 and Tyr123 within the PD-L1 dimer.

Hydrophobic interactions were also frequently encountered and often driven by long alkyl chain tails such as pentyl, hexyl and isopropyl groups, phenyl, methylphenyl and biphenyl systems, and methoxy and methyl groups, particularly those found at the terminal ends of ligand scaffolds. The ether group found at the center of most compounds can potentially be accountable for conformational variability with some degree of polarity appropriate to certain binding environments. In addition, halogen substitutions on phenyl rings such as fluoro, chloro or difluoro groups were frequently encountered. These can enable enhancements of metabolic stability, lipophilicity and binding affinity through halogen bonding or modulation of π-electron density. In one ligand, from (113), a difluoromethyleneoxy group was positioned adjacent to a phenyl ring, where one fluorine atom participated in π-π stacking, while the second fluorine engaged in a non-classical interaction of C-F. . . . C=O, likely contributing to local stabilization through dipolar and electrostatic effects.

Additional substituents of interest included benzodioxan and ethyleneoxy motifs. The benzodioxan ring, via its rigid aromatic framework and embedded ether bridges, contributes primarily to hydrophobic interactions and spatial complementarity within the binding pocket. In contrast, ethyleneoxy linkers, being more flexible and polar, may improve conformational adaptability and form hydrogen bonds via ether oxygen atoms. Collectively, these structural elements define high affinity ligands that effectively mimic key interactions at the PD-L1 interface

From a drug design perspective, these findings highlight the value of integrating aromatic systems to facilitate π stacking, hydrogen bond capable groups, flexible linkers like ethers, and strategic halogenation in order to improve binding affinity and specificity. The observed substituents provide a framework for designing next generation PD-L1 inhibitors with improved pharmacodynamics properties and target engagement.

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CONCLUSION AND PERSPECTIVE

This thesis provides a systematic a thorough meta-analysis of computational studies focused on identification and characterization of Small Molecule Inhibitors of the PD-L1 immune checkpoint, a valuable target in cancer immunotherapy. Tumors exploit the PD-1/PD-L1 interaction as a major immune evasion mechanism, and inhibition of this interface with small molecules offers a complementary or alternative to monoclonal antibody based therapies. Through the condensation of data from several Molecular Docking studies, this research attempted to identify recurring interaction patterns, structural patterns of high affinity ligands and key amino acid residues involved in PD-L1 inhibition. Ultimately, this analysis seeks aims to give the basis for structure-based rational design of future PD-L1 inhibitors.

The meta-analytic forest plot yielded a statistically significant effect ($Z= 8.31$, $p < 0.00001$, 95% CI= [-19.67, -12.16]), confirming that the differences observed across studies were unlikely to be due to pure chance. However, the high heterogeneity ($I^2 = 96\%$) across studies highlights the need for more consistent methodologies in computational drug discoveries.

PDB ID analysis showed that PDB 5N2F was most preferred due to its high quality with a resolution of 1.70 Å. Despite some variability, RMSD values for the majority of ligands remained within the acceptable limit, though some outliers highlighted the need for more consistent docking validation.

The analysis of recurring residues and interaction types highlights key structure-activity relationships (SARs), revealing the importance of amino acids, particularly, Asp122, Ala121 and Gln66, in stabilizing ligand binding through hydrogen bonds, and Tyr56 and Tyr123, in π-π stacking interactions. Structural features such as phenyl, biphenyl rings, chromenone systems, flavonoid backbones and polycyclic systems were routinely found to be involved with π-π stacking, while amines, amides (-NH-), hydroxyl (-OH), carbonyl (C=O), nitriles (C≡N) and ether moieties contribute significantly to hydrogen bonding. Additionally, hydrophobic contacts, frequently mediated by bulky groups like phenyl, biphenyl, isopropyl, pentyl tails and difluorophenyl groups, played a supporting role in anchoring ligands within the PD-L1 binding site. Halogen atoms, especially fluorine, on aromatic rings increased affinity by enhancing both lipophilicity and specific interactions such as rare π-F interactions and C-F…..C=O contacts as seen in difluoromethyleneoxy substituents.

The addition of specialized motifs like benzodioxan and ethyleneoxy groups appeared to offer positioning and flexibility. Ether based groups, often centrally positioned on ligands, likely improve ligand orientation by acting as flexible linkers, enabling optimal alignment of terminal groups with interaction hot spots. Their polarity can also assist in water-mediated or direct hydrogen bonding. Benzodioxan moieties, on the other hand, offer rigidity and electronic content, helping favorable π interactions while occupying hydrophobic pockets.

Looking ahead, future studies should focus on standardizing computational protocols, such as docking settings, scoring functions and RMSD thresholds, in order to increase comparability across datasets. Beyond static docking scores, incorporation of dynamic simulations may yield deeper insights into binding stability. More accurate estimates of binding affinity could also be obtained by integrating quantum mechanical or free energy perturbation (FEP) calculations, particularly for the best performing ligands found in this analysis.

From a design standpoint, these findings underline key structure-activity relationships (SARs) that can guide future inhibitor development. Recurring structural features such as halogenated phenyl rings, polycyclic backbones, heteroatomic systems, alkyl tails and ether linked motifs, such as benzodioxan and ethyleneoxy, are promising scaffolds for PD-L1 inhibitors in the future. Emphasis should also be placed on groups that maintain strong hydrogen bonding with conserved residues, the hydrogen bond donors and acceptors. Planar aromatic systems should also be incorporated as they are involved in π-π stacking interactions with aromatic residues with the PD-L1 interface. Experimental validation of lead compounds, along with ADMET profiling, should be prioritized to transition these compounds from just computational leads to viable anticancer treatments.

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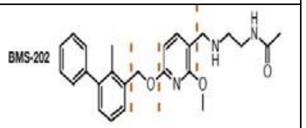
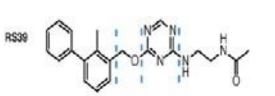
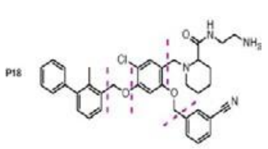
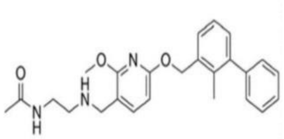
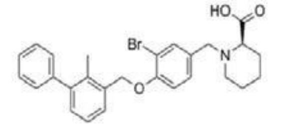
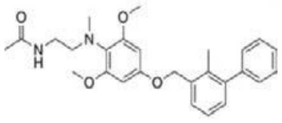
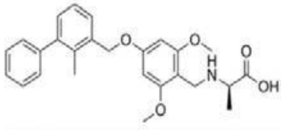
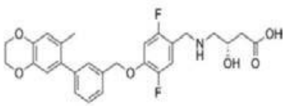
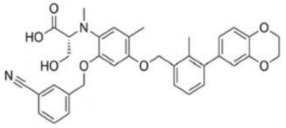
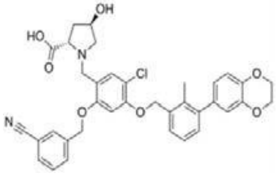
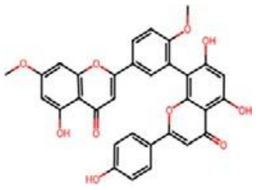
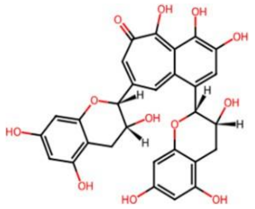
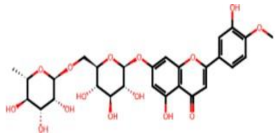
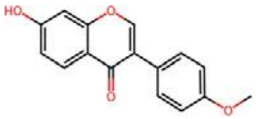
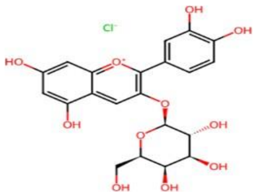
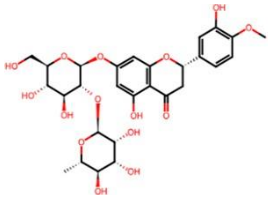
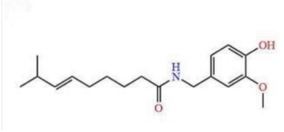
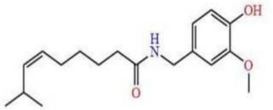
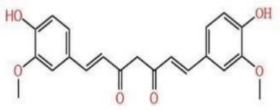
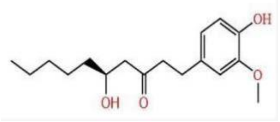
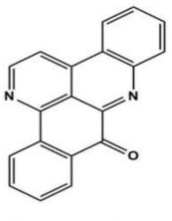
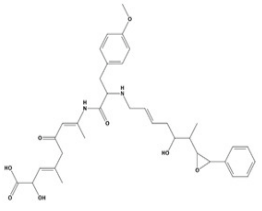
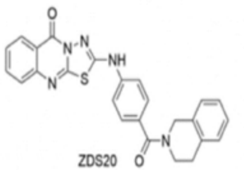
APPENDICES

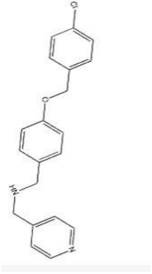
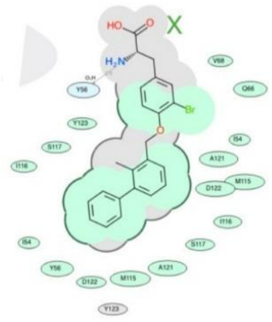
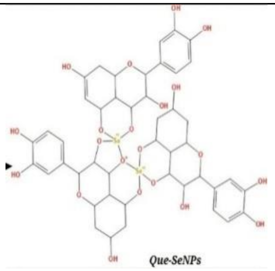
Appendix I: Table summarizing the structures, target. Amino acids, PDB ID, RMSD and Free Binding Energies across different studies

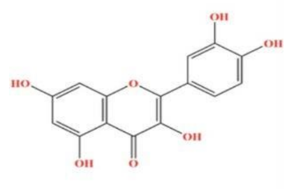
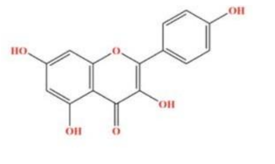
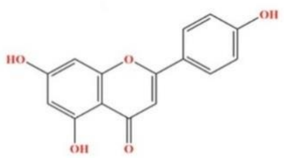
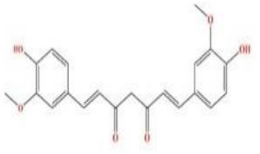
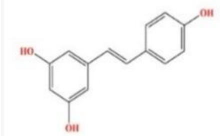
Article	Ligand	Structure	Target	Interaction Amino Acids	PDB ID	RSMD	Energy(-kcal/mol)	Cancer
1. Donati et al., 2023	Biphenyl based inhibiters		PD-L1					
	BMS-202			Thr20, Ile54, Tyr56, Met115, Ser117, Ala121, Asp122, Tyr123, Lys124,Val76, Arg113, Gln66, Glu58	5J89, 5J89, 6R3K	1.5 Å	(-)	melanoma, breast, pancreatic, renal and non-small cell lung (NSCLC) carcinomas
	RS39			Thr20, Ile54, Tyr56, Met115, Ser117, Ala121, Asp122, Tyr123, Val76, Arg113, Gln66, Glu58			(-)	
	P18			Thr20, Ile54, Tyr56, Met115, Ser117, Ala121, Asp122, Tyr123, Arg125, Val76, Asp73, Gln66, Arg113, Glu58			(-)	
2. Kuang et al., 2020	BMS inhibitors		PD-L1					
	BMS-202			Tyr56, Met115, Ala121, Asp122, Tyr123, Lys124	5J89	2.39 Å	(-)	

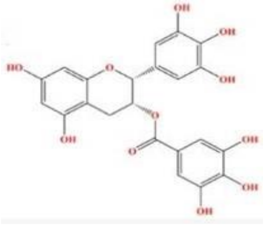
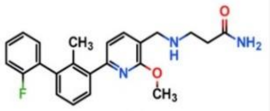
	BMS-8			Tyr56, Met115, Ala121, Asp122, Tyr123, Lys124	5J8O	2.08 Å	(-)	
	BMS-37			Tyr56, Met115, Ala121, Asp122, Tyr123, Lys124	5N2D	1.4 Å	(-)	
	BMS-105			Tyr56, Met115, Ala121, Asp122, Tyr123, Lys124	6NM8	1.57 Å	(-)	
	BMS-200			Tyr56, Met115, Ala121, Asp122, Tyr123, Lys124	5N2F	1.33 Å	(-)	
	BMS-1001			Tyr56, Met115, Ala121, Asp122, Tyr123, Lys124	5NIU	1.27 Å	(-)	
	BMS-1166			Tyr56, Met115, Ala121, Asp122, Tyr123, Lys124	6R3K	2.09 Å	(-)	
3. Jiang et al., 2025	Flavanoids		PD-L1		7DY7			non-small-cell lung cancer, melanoma, bladder carcinoma, and gastric cancer
	(A) Ginkgetin			Gln49, Asn46, Arg108, Met98, Ile37, Ser100, Asp105		2.78 Å	46.73	

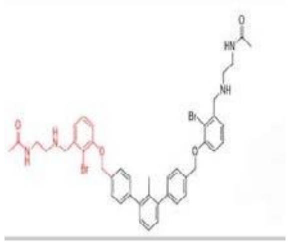
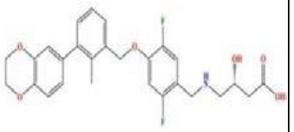
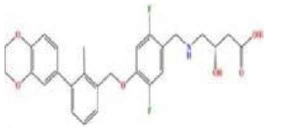
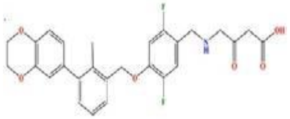
	(B) Theaflavin			Ala1, Gly103, Ala104, Ser100, Gln49, Met98, Asn46		2.58 Å	37.45	
	(C) Diosmin			Glu54, Gln49, Phe2, Ala104, Thr3, Asp105		2.41 Å	44.96	
	(D) Formononetin			Gln49, Ile99, Met98		2.28 Å	40.9	
	(E) Idaein			Met98, Gln49, Asp105, Phe2, Ala104, Gly103		2.26 Å	44.47	
	(F) Neohesperidin			Asp56, Gln49, Tyr39, Phe2, Thr3, Ala104, Lys107		2.25 Å	40.3	
4. X. Wu et	Food-Derived Small Molecules		PD-L1		5N2F			(-)

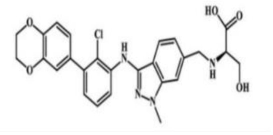
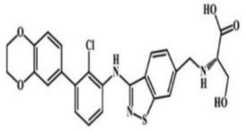
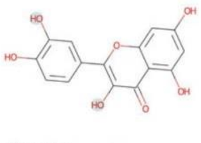
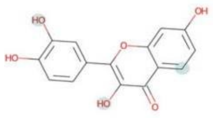
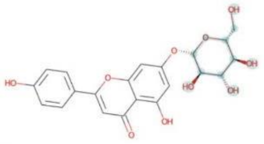
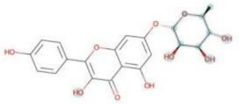
al., 2023	Capsaicin			Tyr123, Ala121, Met115, Tyr56, Ile54, Gln66		2.71 Å	42.53	
	Zucapsaicin			Tyr56, Ile54, Tyr123, Asp122, Ala121, Gln66, Met115		2.41 Å	43.75	
	Curcumin			Ile54, Tyr56, Met115, Tyr123, Ala121		2.61 Å	39.19	
	6-Gingerol			Ile54, Tyr56, Val68, Met115, Ala121, Tyr123, Gln66, Ser117		2.49 Å	39.2	
5. Ouchaoui et al., 2024	Benzosampangine		PD-L1	Met115, Tyr56, Ala121, Ser117, Tyr123, Asp122, Ile116, Ile54, Gln66.	5O45	1.52Å	39.39	metastatic NSCLC, melanoma, urothelial carcinoma, and Merkel cell carcinoma.
6. Luo et al., 2021	Compound 51320		PD-L1	Tyr123, Met115, Ile54, Asp122, Ala121, Ser117, Ile116, Try56, Gly120, Ala18, Phe19	6R3K	(-)	37.04	lung cancer
7. T. Wu et al., 2024	ZDS20		PD-L1	Ile54, Tyr56, Met115, Ala121, Tyr123, Ile116, Ser117, Val68, Gln66, Asp122, Lys124	6R3K	0.5-1.5 Å	33.02	(-)

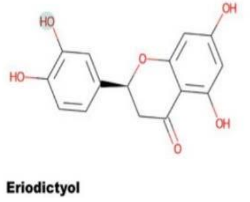
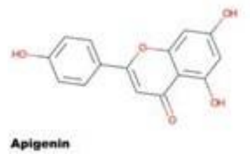
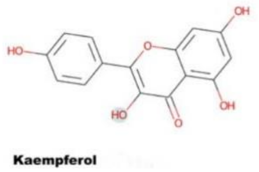
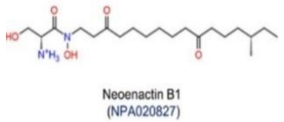
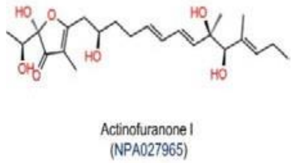
8. Wang et al., 2023	CBPA (N-{4-[(4-chlorobenzyl) oxy]benzyl}N-(4-pyridinylmethyl)amine)		PD-L1	Thr20, Asp122, Tyr123, Met115, Ser117, Ile116, Ile54, Ala121, Val68, Tyr56, Gln66, Val55	5J89	(-)	24.63	melanoma, bladder cancer, metastatic non-small-cell lung cancer, and skin Merkel cell carcinoma
9. Kim et al., 2020	X (biphenyl-conjugated bromotyrosine)		PD-L1	Ile54, Tyr56, Val68, Met115, Ile116, Ser117, Ala121, Asp122 , Tyr123, Gln66.	5J8O	0.40 Å	(-)	(-)
10.Abd ul-Razek et al., 2024	Quercetin-Selenium Nanoparticles		PD-L1	(Tyr123,Asp122, Phe19), Arg125, Ala121, Thr20, Lys124, Ala18	5NIU	(-)	(-)	gastric, colorectal, breast, renal, and non-small cell lung cancers
	Flavonoid Compounds		PD-L1	Ile54, Tyr56, Met115, Ala121, Tyr123.	5N2F			(-)

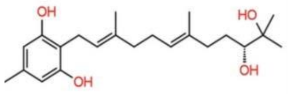
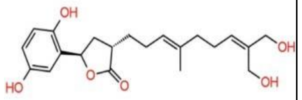
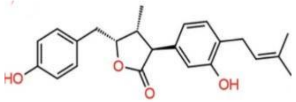
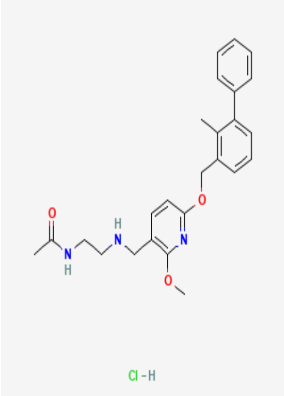
11.Guo et al., 2024	Quercetin(Que)			Ile54, Tyr56, Ile116, Ser117, Asp122, Met115, Ala121, Gln66, Tyr123, Val55		2.31 Å	35.39	
	Kaempferol(Kmp)			Ile54, Tyr56, Met115, Ile116, Ser117, Ala121, Tyr123, Asp122		2.38 Å	32.66	
	Apigenin(Api)			Ile54, Tyr56, Tyr123, Met115, Lys124, Ala121, Ile116, Ser117, Val55, Gln66		3.11 Å	21.49	
12. Guo et al., 2021	Food-Derived Polyphenols		PD-L1	Ile54, Tyr56, Met115, Ala121,Tyr123	**5N2F**			non-small-cell lung cancer, oral and ovarian cancer
	Curcumin(CC)			Ile54, Tyr56, Met115, Ala121, Tyr123, Asp122, Ser117, Gln66	4K58	2.30 Å	33.72	
	Resveratrol(RSV)			Tyr56, Tyr123, Ile54, Met115, Ile116, Ala121	1CGZ	2.44 Å	28.72	oral and ovarian cancer cells

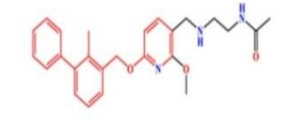
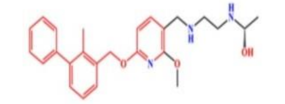
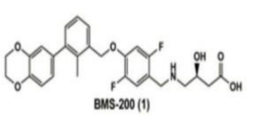
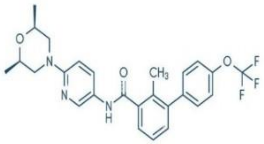
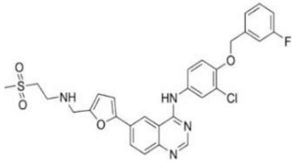
	Epigallocatechin gallate (EGCG)			Tyr123, Lys124, Phe19, Asp122, Ala121, Thr20, Met115, Tyr56, Gln66, Ile54, Val68	3NG5	2.90 Å	20.31	non-small-cell lung cancer cells
13. Alanzi et al., 2024	Compound-49642753		PD-L1		5N2F			(-)
				Ile54, Tyr56, Met115, Ala121, Tyr123, Asp122		(-)	(-)	
	Compound-857389			Ile54, Tyr56, Met115, Ala121, Tyr123		(-)	(-)	
14. Zhang et al., 2021	**(compound A9)** 2-(2-methyl-[1,1′-biphenyl]-3-yl) pyridine derivatives	 A9	PD-L1	Asp122, Tyr123, Arg125, Tyr56	5J89	(-)	(-)	(-)

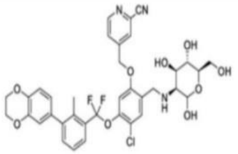
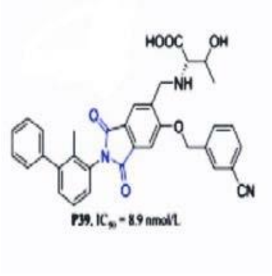
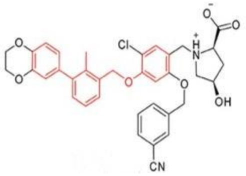
15. Klimek et al., 2024	C2-Symmetrical Terphenyl Derivatives				6VQN			
	Compound 7j		PD-L1	Met115, Ala121, Tyr123, Ile54, Ile116, Tyr56, Lys62, Ala18, Phe19, Thr20, Val21, Ser117, Val55, Asp122, Gln66, Asp61, Lys124, Arg125		(-)	(-)	(-)
16. Guo, Jin, et al., 2021	BMS-200-related small-molecule inhibitors		PD-L1	Ile54, Tyr56, Met115, Ala121 and Tyr123	5N2F			
	(MOD)-BMS-200			Ile54, Tyr56, Met115,Tyr123, Val68, Ala121		(-)	42.45	
	(S)-BMS-200			(Ile54, Tyr56, Met115, Val68, Ala121, Tyr123), Gln66		(-)	42.42	
	(R)-BMS-200			Ile54, Tyr56, Met115, Tyr123, Val68, Ala121.		(-)	40.48	
			PD-L1		6R3K			Lung cancer

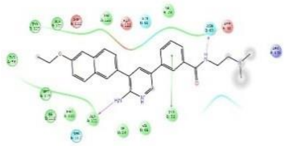
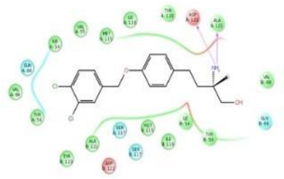
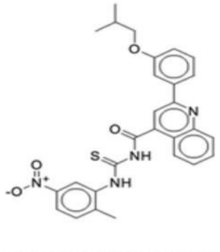
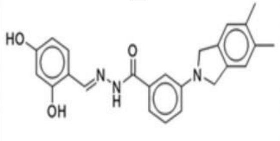
17. Zhai et al., 2025	molecule 58			Tyr123, Met115,Val55, Ile54, Ala121, Tyr56, Ile116, Gln66, Thr20, Asp122		(-)	(-)	
	molecule 34			Tyr123, Met115, Val55, Ile54, Ala121, Tyr56, Ile116, Val68, Gln66, Thr20, Asp122		(-)	(-)	
18. Sartori et al., 2022			PD-L1	((Tyr56, Glu58, Asp122, Lys124, and Arg125))	5N2F, 6NM7,6VQN,7MLD			urothelial carcinoma or metastatic urothelial carcinoma, non-small cell lung, and prostate cancer
	Quercetin	 Quercetin		Tyr123, Asp122, Ile116, Gln66, Tyr56, Ala121, Ile54, Met115, Asn63, Glu58, Arg125, Ser117		3.4 Å	32.4	
	Fisetin	 Fisetin		Asp122, Ile116, Met115, Tyr56, Ile54, Gln66, Ala121, Ser117, Arg125, Glu58			33.1	
	Cosmosiin	 Cosmosiin		Arg125, Lys124, Glu58, Tyr123, Ile116, Gln66, Asp61, Tyr56, Asp122		3.1 Å	50.2	
	Kaempferol-7-O-rhamnoside	 Kaempferol-7-O rhamnoside		Asp61, Tyr123, Asn63, Asp122, Tyr56, Gln66, Ile54, Ser117, Lys124, Arg125, Glu58		4.2 Å	39.9	

	Eriodictyol			Asp122, Met115, Tyr56, Ile116, Gln66, Ile54, Ser117, Lys124, Arg125, Glu58		3.6 Å	27.8	
	Apigenin			Asp122, Met115, Ala121, Tyr56, Ser117, Gln66, Ile54, Lys124, Arg125, Glu58		3.1 Å	34.7	
	Kaempferol			Asp122, Met115, Ile116, Tyr56, Gln66, Val68, Asp73, Ile54, Ser117, Lys124, Arg125, Glu58		12.9 Å	33.9	
19. Kumar et al., 2022			PD-L1		6R3K			(-)
	Neoenactin B1			Tyr56, Ile54, Met115, Ile116, Ser117, Tyr123, Asp122, Ala121, Lys124, Gln66, Asn63, Asp61, Glu58		(-)	73.55	
	Actinofuranone I			Ser117, Ile116, Met115, Ile54, Tyr56, Ala121, Asp122, Tyr123, Lys124, Asn63, Val76, Gln66, Val68, Thr20, Phe19, Ala18, Val55		(-)	81.07	

	Cosmosporin A	 Cosmosporin A (NPA026024)		Tyr123, Met115, Ile116, Ala121, Asp122, Lys124, Asp61, Gln66, Ser117, Ile54, Tyr56		(-)	83.96	
	Ganocapenoid A	 Ganocapenoid A (NPA026082)		Tyr56, Ile54, Met115, Ile116, Ser117, Gln66, Val68, Ala121, Asp122, Tyr123.		(-)	84.56	
	[3-hydroxy-4-(3-methy/lbut-2-enyl)phenyl]-5-(4-hydroxybenzyl)-4-methyldihydrofuran-2(3H)-one	 3-[3-hydroxy-4-(3-methylbut-2-enyl)phenyl]-5-(4-hydroxybenzyl)-4-methyldihydrofuran-2(3H)-one (NPA013736)		Lys124, Tyr123, Asp122, Ala121, Ser117, Ile116, Met115, Ile54, Val55, Tyr56, Gln66		(-)	87.21	
20. Udhwani et al., 2019	XGIQBUNWFCCMAS-UHFFFAOYSA-N (PubChem CID: 127263272)		PD-L1	Val55, Asp122, Ile54, Ala121, Ser117, Ile116, Phe67, Val68, Ala18, Gly120, Tyr56, Tyr123, Met115, Phe19, Gln66, Thr20	5J8O	(-)	(-)	Lung cancer

21. Liang et al., 2023			PD-L1		5N2F, 5J89			(-)
	BMS-200			Tyr123, Tyr56, Met115, Ala121, Ile54, Gln66, Asp73		(-)	42.32	
	BMS-202			Tyr56, Met115, Alal121, Tyr123, Gln66, Asn63		(-)	43.17	
	MS system			Tyr56, Met115, Tyr123, Ala121, Gln66, Asn63, Val76		(-)	45.7	
	MR system			Tyr56, Ala121, Met115,Tyr123, Gln66		(-)	44.13	
22. S. Sobral et al., 2025			PD-L1		5N2F, 5J89			(-)
	BMS-200	 BMS-200 (1)		Ile54, Tyr56, Met115, Ala121, Tyr123, Lys124, Asp122		(-)	(-)	
	Sonidegib			Ile54, Tyr56, Met115, Ala121, Tyr123, Gln66, Asp122		(-)	(-)	
	Lapatinib			Tyr56, Met115, Ala121, Tyr123, Asn63, Gln66, Asp122		(-)	(-)	

23. Song et al., 2021	compound 43		PD-L1	Tyr56, Asp122, Lys124, Gln66, Arg125	5NIU	(-)	(-)	(-)
24. Sun et al., 2022	(Pthalamide derivative) compound P39		PD-L1	Tyr 56, Ala 121, Met 115, Arg125, Asp 122, Lys 124	5J89	2.7 Å	(-)	(-)
25. Shi et al., 2019		 	PD-L1	(Ile54, Tyr56, Met115, Ala121, Tyr123, Lys124)	5J8O, 5NIX			(-)
	BMS-8			Tyr56, Met115, Ala121, Tyr123, Ala54, Gln66, Ile54, Lys124		(-)	29.6	
	BMS-1166			Ile54, Tyr56, Met115, Ala121,Asp122,Tyr123, Arg125, Val76, Lys124		(-)	42.67	
26. Patil et			PD-L1	(Tyr56, Asp122, Lys124, Arg125)	5N2F			(-)

al., 2022	CRT5			Met115, Ile116, Ser117, Ala121, Ile54, Val68, Tyr56, Arg125, Asp61, Asn63, Val76, Gln66, Tyr123, Asp122, Lys124		2.0 Å	(-)	
	P053			Met115, Ile116, Se117, Ala121, Ile54, Val68, Val55, Tyr56, Asp122, Gln66, Tyr123, Arg125, Lys124		3.0 - 4.0Å	(-)	
27. Kamal et al., 2023	compound (H6)	H6 	PD-L1	Ile54, Tyr56, Met115, Ser117, Ala121, Asp122, Tyr123, Glu58, Lys124, Arg125, Gln66, Val68, and Val76	5J8O	1.124 Å	54.8842	melanoma, head and neck, lung, bladder and Hepatocellular cancers
	compound (H4)	H4 		Val76, Asn63, Gln66, Tyr56, Ile54, Arg125, Tyr123, Ala121, Asp122, Met115, Ser117, Lys124			42.4763	

ABSTRACT:

Background: PD-L1(Programmed Death Ligand-1) is a key immune checkpoint target in anticancer therapy. Many small molecule inhibitors have been proposed via in silico studies, but their comparative performance analysis remains lacking.

Objective: To conduct a meta-analysis of PD-L1 inhibitors based on binding affinities, interaction patterns and structural features.

Methods: Data from 27 articles covering 69 ligands were analyzed. Variables included binding free energies, RMSD values, molecular interactions and key amino acids. A forest plot was constructed to statistically evaluate binding affinity variations.

Results: The forest plot showed a statistically significant difference in binding energies ($Z = 6.09$, $p < 0.00001$), confirming non-random performance among top ligands. Common residues included Tyr56, Met115 and Ala121. Frequent features were hydrogen bonds, π-π stacking and hydrophobic interactions.

Conclusion: This work identifies key interaction motifs and substituents for potent PD-L1 inhibition, supporting their use in future structure-based drug design.

Keywords: PD-1/PD-L1, docking, molecular interaction, anticancer therapy, in silico drug design.

RÉSUMÉ :

Contexte : Le PD-L1 (Programmed Death Ligand-1) est une cible immunitaire clé dans la thérapie anticancéreuse. De nombreux inhibiteurs de petite taille ont été proposés à travers des études in silico, mais une analyse comparative de leurs performances fait encore défaut.

Objectif : Réaliser une méta-analyse des inhibiteurs de PD-L1 basée sur les affinités de liaison, les schémas d'interaction et les caractéristiques structurales.

Méthodes : Les données de 27 articles portant sur 69 ligands ont été analysées. Les variables étudiées incluaient les énergies libres de liaison, les valeurs RMSD, les interactions moléculaires et les acides aminés clés. Un diagramme en forêt a été construit pour évaluer statistiquement les variations des affinités de liaison.

Résultats : Le diagramme en forêt a révélé une différence significative des énergies de liaison ($Z = 6,09$, $p < 0,00001$), confirmant que les performances observées parmi les meilleurs ligands ne sont pas dues au hasard. Les résidus fréquemment impliqués comprenaient Tyr56, Met115 et Ala121. Les motifs d'interaction les plus courants étaient les empilements les liaisons hydrogène, π-π et les interactions hydrophobes.

Conclusion : Ce travail met en évidence des motifs d'interaction clés et des substituants favorables à une inhibition efficace de PD-L1, soutenant leur intégration dans la conception future de médicaments basés sur la structure.

Mots-clés : PD-1/PD-L1, docking, interaction moléculaire, thérapie anticancéreuse, conception de médicaments in silico.

الملخص:

الخلفية :يُعدّ (PD-L1 الليغاند 1 للموت المبرمج) هدفًا رئيسيًا لنقاط التفتيش المناعية في علاج السرطان. وقد اقترحت العديد من الدراسات الحاسوبية (*in silico*) مثبطات صغيرة للجزيءPD-L1 ، لكن لا تزال التحليلات المقارنة لأدائها محدودة.

الهدف :إجراء تحليل تلوي لمثبطات PD-L1 اعتمادًا على طاقات الارتباط، وأنماط التفاعل، والخصائص البنيوية.

الطرق :تم تحليل بيانات مستخلصة من 27 مقالة علمية شملت 69 جزيئًا. تضمنت المتغيرات المدروسة طاقات الارتباط الحرة، قيمRMSD ، التفاعلات الجزيئية، والأحماض الأمينية الرئيسية. كما تم بناء مخطط غابة (*forest plot*) لتقييم الفروقات في طاقات الارتباط من الناحية الإحصائية.

النتائج :أظهر مخطط الغابة وجود فرق معنوي في طاقات الارتباط($Z = 6.09$ ، $p < 0.00001$)، مما يؤكد أن الأداء بين أفضل الجزيئات لم يكن عشوائيًا. من بين الأحماض الأمينية الأكثر تكرارًا في التفاعل: Tyr56 و Met115و Ala121. أما أبرز أنماط التفاعل فشملت التكدس الروابط الهيدروجينية، وπ–π والتفاعلات الكارهة للماء.

الاستنتاج :يحدد هذا العمل أنماط التفاعل الرئيسية والمجموعات الفعالة اللازمة لتثبيطPD-L1 ، مما يدعم استخدامها في تصميم أدوية مستقبلية قائمة على البنية.

الكلمات المفتاحية: PD-1/PD-L1، الالتحام الجزيئ ، التفاعل الجزيئي، العلاج المضاد للسرطان، تصميم الأدوية الحاسوبي.